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ERRATA AND AUTHORS' EMENDATIONS

- Page 41, fifteenth line: "*G*" should be "*I*."
- Page 60, table 1, block D, column D, block E, column E, block F, column F, block G, column G, and block H, column H. Last figure "1.0000" should be "-1.0000."
- Pages 60 and 64, tables 1 and 9, the three lines of all blocks below the first block are spoken of as line 1, 3, and 4.
- Page 61, table 5, block F, column F: Last figure should be "-1.0000."
- Page 63, table 8, block I, column G, first line: "0.01456" should be "0.1456"; and block E, column D, fourth line, "1858" should be "1853."
- Page 64, table 9: The brackets in block I, column H, should include the first two lines and the brackets in block H, column H, should include the last three lines.
- Page 64, table 9: Instead of beta "AC" in column B insert "AB", and in column A omit "AB."
- Page 65, twenty-first line from bottom: "0.944" should be "0.9994."
- Page 86, second line: "fig. 3, *C*, *f*" should be "fig. 3, *C*, *b*."
- Page 86, fifteenth line: "fig. 4, *C*, *d*" should be "fig. 4, *C*, *c*."
- Page 136, legend for plate 1: "*E*" should be "*G*", "*F*" should be "*E*"; and "*G*" should be "*F*."
- Page 137, footnote: Between "percent" and "solution" insert "aqueous" and between "solution" and "to" insert "of phenol."
- Page 139, sixteenth line: "pl. 1, *E*" should be "pl. 1, *G*"; twenty-first line, "Plate 1, *F*" should be "Plate 1, *E*"; and thirty-first line, "pl. 1, *G*" should be "pl. 1, *F*."
- Page 145, table 1, last column: The cross rule over "Diameter of sorus" should be omitted and the vertical rule at left of column should continue to top.
- Page 189, bottom of page: "Key No. G-915" should be "Key No. G-951."
- Page 198, line 17: Should be
$$\begin{array}{cc} \text{Covariance } us & S(us - \bar{u}\bar{s}) \\ \text{Variance } s & \text{or } S(u - \bar{u})^2 \end{array}$$
- Page 204, fourteenth line: "increase" should be "decrease"
- Page 318, ninth line from bottom: "a day's" should be "10 days'."
- Page 322, table 20, columns 15 and 16, third deck boxhead: After "44 days" should be "44 days²."
- Page 346, table 10, columns 3 and 6: " $r \pm 0$ " should be " $r \neq 0$."
- Page 401, table 7, under Victoria (susceptible), second column: "50" should be inserted in blank space.
- Page 484, legend for table 1: "Alaska" should be "Alysia" and "Uralde" should be "Uvalde."
- Page 550, eleventh line from bottom: Eliminate "an awned fertile lemma."
- Page 551, Literature Cited, reference (3): "123 pp." should be "23 pp."
- Page 607, eleventh line: "(25)" should be "(26) "
- Page 741, twenty-second line: "had" should be omitted, and between "germinated" and "at" should be inserted "in the sample taken."
- Page 829, ninth line from bottom: Omit "(pl. 2, *C*)."
- Page 837, first and second lines: The superscript 6 in the formulas should be a subscript 6.
- Page 850, table 2, footnote 2: Should be $\sqrt{\frac{\sum x^2}{N}} - \bar{x}^2$.
- Page 864, fourteenth line from bottom: "*tritic*" should be inserted after "*gramin*ts."
- Page 868, twelfth line from bottom: "forms" should be "varieties."

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No. 1

THE UTILIZATION OF ENERGY-PRODUCING NUTRI-MENT AND PROTEIN IN WHITE AND YELLOW CORN AND IN DIETS DEFICIENT IN VITAMINS A, D, AND G¹

By WINFRED W. BRAMAN, *associate professor of animal nutrition*; ALEX BLACK, O. J. KAHLBERG, and LEROY VORIS, *instructors in animal nutrition*; R. W. SWIFT, *assistant professor of animal nutrition*, and E. B. FORBES, *director, Institute of Animal Nutrition, Pennsylvania Agricultural Experiment Station*

A COMPARISON OF THE NUTRITIVE VALUE OF YELLOW AND WHITE CORN²

Many feeding experiments have been conducted for the purpose of comparing the efficiency of white and yellow corn in providing the requirements of maintenance and growth of animals; but in few of these experiments has the composition of the body increase been determined, and in none has there been a complete accounting for the food energy.

REVIEW OF LITERATURE

Steenbock and Boutwell (32)³ compared two rations containing 85 percent of yellow dent corn and 85 percent of white corn, respectively. They found that the yellow corn ration gave from good to normal growth, while the white corn ration failed to promote growth, and in some cases even failed to provide the requirements of maintenance. In the one case in which a white corn ration appeared to produce growth close inspection revealed the presence of some yellow pigment in the white corn.

Steenbock and Coward (33) demonstrated the superior value, for growth, of yellow corn over white corn, and also located the vitamin A in the pigment of the endosperm.

Fraps (8) compared varieties of corn, as to vitamin A content, in experiments with rats, by Sherman and Munsell's (29) unit method, with preliminary depletion of the subjects as to vitamin A, and also by a so-called "ration method", in the use of which there was no preliminary depletion of the rats, the vitamin A being measured by the gain in weight of the animals.

The unit method showed 1 g of yellow corn to contain 2.5 to 8 units per gram, while the vitamin A in most of the samples of white corn was very low—1 unit in 25 to 35 g. Some samples of white corn, however, contained 1 unit in 2 to 5 g of corn—which was considered as possibly resulting from crossing with yellow corn. Season and soil appeared to have some effect on vitamin values.

¹ Received for publication July 25, 1934; issued April 1935. Technical paper no. 648.

² The studies of the comparative nutritive value of yellow and white corn were conducted by Braman, Black, Voris, and Forbes; those on the effects of vitamin A deficiency (pp. 13-19) by Braman, Black, and Forbes; those on the effects of vitamin D deficiency (pp. 19-26) by Braman, Black, Swift, and Forbes; and those on the effects of vitamin G deficiency (pp. 26-34) by Swift, Kahlenberg, and Forbes.

³ Reference is made by number (italic) to Literature Cited, p. 36.

Russell (26) found yellow dent corn to be about 50 percent richer in vitamin A than white-capped yellow dent corn.

Hauge, as reported by Skinner (30), studied three varieties of corn, Woodburn, with deep yellow endosperm, Reid Yellow Dent, with medium yellow endosperm, and Clement, which contained a smaller amount of yellow. The content of vitamin A varied with the depth of the yellow in these varieties.

Hauge and Trost (14), in studies of inheritance of vitamin A in maize, found it to be transmitted exclusively with the yellow endosperm, through the process of crossing and segregation.

Palmer (23) and Palmer and Kennedy (24) investigated the carotinoids and their relation to growth and reproduction in rats. They concluded that the rat itself contains no carotinoids. Growth and reproduction of rats were maintained on a diet in which the sole source of fat-soluble vitamin was ewe's milk fat containing only 0.00014 percent carotene. Carotinoid-free egg yolk, as the sole source of vitamin A, also served the requirements of growth and reproduction in rats.

Potter (25) found that the McIntosh apple, which has white flesh, has a vitamin A potency at least equal to that of two varieties with yellow flesh—Red Delicious and Golden Delicious—and concluded, therefore, that the presence of yellow pigment in the apple is not related to vitamin A potency.

The invariable presence of a yellow pigment with vitamin A, therefore, is not accepted as a fact. Further, the yellow pigment in a feeding stuff is sometimes so located that casual observation does not reveal its presence. The pigment in corn is located in the endosperm, but the outside of the kernel may be white, and may obscure this yellow color. Even when the corn is finely ground the pigment may not be in evidence to superficial inspection.

Kick and Bethke (15), in experiments on the effects of storage of corn, found very little if any destruction of vitamin A even when whole, cracked, or finely ground yellow corn was kept for as long as a year.

Practically all of the measurements of vitamin A value, especially by the use of rats, have had as their criterion simply the increase in growth of the animals; but Mitchell and Carman (21) went further and studied the utilization of food energy in growing rats and the composition of the gains in weight. They showed that—

a gain in weight of the live rat, even for a definite age, from a definite initial weight and during a feeding period of definite length, is not a definite measure of the nutritive effect of the food.

Sampson and Korenchevsky (28) raised a question as to whether the changes produced in body weight and in the deposition of fat, especially with male rats, on a vitamin A deficient diet, was due to this deficiency alone or to a resulting decrease in appetite and in food consumption. They concluded that vitamin A has both an appetite-producing and an anabolic effect.

The object of the experiment to be discussed was to compare the energy and nitrogen metabolism of rats on two rations of the same gross energy content and of the same gross composition, except that one ration contained yellow and the other white corn.

PRELIMINARY EXPERIMENT

A first trial, of incomplete character, was made with yellow corn and white corn which were purchased locally, but were of unknown varie-

ties and origin. After the corn was ground, the yellow corn meal was decidedly yellow, while the white corn meal was dead white in color.

The composition of the rations was as follows:

YELLOW CORN RATION		WHITE CORN RATION	
	Percent		Percent
Yellow corn.....	70.00	White corn.....	70.00
Crisco.....	10.00	Crisco.....	10.80
Starch.....	3.00	Gelatin.....	.29
NaCl.....	1.00	Starch.....	1.91
Osborne and Mendel salts.....	4.00	NaCl.....	1.00
Casein (vitamin-A-free).....	12.00	Osborne and Mendel salts.....	4.00
	100.00	Casein (vitamin-A-free).....	12.00
			100.00

The yellow corn ration contained 2.87 percent N (17.94 percent protein) and 4,276 calories per gram, while the white corn ration contained 2.89 percent N (18.06 percent protein) and 4,375 calories per gram.

Vioosterol of a potency of 250 D was added to each ration in the amount of 0.00066 g per 100 g of other components; and one tablet of Harris yeast vitamin (weighing on an average 0.1112 g) was fed daily to each rat.

The casein used was Harris casein, vitamin-B-free. This was washed, to remove vitamin A, by boiling in 95-percent alcohol, for 2 periods of 4 hours each, after which it was dried for 4 days in an oven. The vitamin A content of the casein was then tested by feeding it, in the following diet, to 4 rats: Casein 18 parts, starch 78 parts, Osborne and Mendel salt mixture 4 parts. Vioosterol and yeast were fed daily apart from the remainder of the diet.

The experimental animals in the corn-feeding experiment were six pairs of weanling albino rats. The members of each pair were of the same age, litter, and sex, and were of about the same weight (40 g) when weaned at 28 days of age.

The rats were fed by the paired feeding method, as described by Swift, Kahlenberg, Voris, and Forbes (34), in the first paper of this series, and use was also made of the same control group of 10 rats, as in the study mentioned, as a basis of computation of the composition of the bodies of the rats at the beginning of this corn-feeding experiment.

One rat of each pair was fed the yellow, and the other the white corn ration. They were fed daily for 13 weeks, the members of each pair of rats receiving the same quantity of feed, and therefore essentially the same quantities of nitrogen and energy.

The rats were kept in individual cylindrical cages, 8 inches in diameter, made of 3-mesh hardware cloth, the cages resting in shallow metal pans. The urine was not saved in this preliminary experiment, but was absorbed by paper toweling in the metal pans. The feces were collected daily, and were analyzed at the end of the experiment as one sample for each rat.

Daily feed records were kept, and the body weight of each rat was recorded every third day. At the end of the 13 weeks' feeding the rats were killed by gas, the body weights were recorded (1) as gross weight, and (2) after the removal of the intestinal contents. The bodies were then dried in vacuum and extracted with ether.⁴

⁴ The work of this preliminary experiment, to the time of extraction of the rats, was performed by R. C. Miller, instructor in animal nutrition at the Pennsylvania station.

The data observed from this group of rats were the ether extract and the energy of the ether extract of the rat bodies, the nitrogen and the energy of the ether-extracted bodies, and the nitrogen and the energy of the feces; and, by subtraction of corresponding data from the control group, which has been mentioned, the gains of the rats which received the diets compared were computed.

In the feeding of these rats there were 116 refusals of feed, 75 by the rats on the white corn ration and 41 by the rats on the yellow corn ration. This deviation of 17 from the normal outcome is 3.15 times the standard deviation of 5.39, and would occur by chance only once in 727 trials. This signifies that the yellow corn ration was definitely the more palatable of the two.

After 92 to 93 days' feeding, the rats on the white corn ration alone manifested symptoms of xerophthalmia, and in another day the experiment was terminated. The data obtained from the 6 pairs of rats are shown in tables 1 to 4.

TABLE 1.—*Gain in live weight¹ of rats fed yellow corn ration and white corn ration as related to dry matter of food*

Pair no.	Rates fed yellow corn			Rates fed white corn		
	Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food
	Grams	Grams	Percent	Grams	Grams	Percent
1	539 0	122 0	22.6	546 9	107 0	19.6
2	610 9	196 0	32.1	619 9	159 0	25.6
3	529.1	127 0	24 0	537.8	113 0	21 0
4	467.6	134 0	29.3	463.4	106 0	22 9
5	484.9	114 0	23 5	494 8	100 0	20 2
6	546.1	182 0	33 3	554 1	140 0	25 3
Average	527 9	145 8	27 5	536 2	120 8	22 4

¹ Contents of alimentary tract included.

TABLE 2.—*Energy of body gain of rats fed yellow corn ration and white corn ration as related to energy of food*

Pair no.	Rats fed yellow corn			Rats fed white corn			Body gain of energy of white corn rats as percentage of gain of pair mate
	Food energy	Body gain		Food energy	Body gain		
		Total	As percentage of food energy		Total	As percentage of food energy	
	Calories	Calories	Percent	Calories	Calories	Percent	Percent
1	2,553	364.3	14.3	2,612	253.1	9.7	69.5
2	2,896	544.6	18.8	2,962	463.8	15.7	85.2
3	2,506	377.4	15.1	2,568	337.8	13.2	89.5
5	2,297	416.8	18.1	2,362	342.4	14.5	82.1
6	2,568	460.9	17.8	2,647	426.6	16.1	92.6
Average	2,568	432.8	16.8	2,630	364.7	13.8	83.8

TABLE 3.—*Nitrogen of body gain of rats fed yellow corn ration and white corn ration as related to fat and energy of body gain and to nitrogen of food*

RATS FED YELLOW CORN RATION								
Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram of nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	Grams	Grams	Calories	Percent	Percent	Grams	Percent
1	4.34	24.7	5.7	364.3	61.9	38.1	17.36	25.0
2	6.58	34.4	5.2	544.6	59.0	41.0	19.66	33.5
3	1.12	25.8	6.3	377.4	62.9	37.1	17.04	24.2
4	4.85						14.76	32.9
5	3.59	32.3	9.0	416.8	71.6	28.4	15.64	23.0
6	6.66	27.5	4.1	460.9	54.9	45.1	17.59	37.9
Average	5.02	28.9	6.1	432.8	62.1	37.9	17.01	29.4

RATS FED WHITE CORN RATION								
Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram of nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	Grams	Grams	Calories	Percent	Percent	Grams	Percent
1	4.26	12.5	2.9	253.1	45.8	54.2	17.48	24.4
2	5.99	28.6	4.8	463.8	57.8	42.2	19.79	30.3
3	4.39	21.3	4.9	337.8	59.1	40.9	17.19	25.5
4	4.08						14.83	27.5
5	3.60	23.9	6.6	342.4	65.4	34.6	15.83	22.7
6	4.59	29.4	6.4	426.6	64.1	35.9	17.71	25.9
Average	4.49	23.1	5.1	364.7	58.4	41.6	17.14	26.1

TABLE 4.—*Digestibility of protein and energy-producing nutriment of yellow corn ration and white corn ration when fed to rats*

Pair no.	Rats fed yellow corn						Rats fed white corn					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Di-gested	Food	Feces	Di-gested	Food	Feces	Di-gested	Food	Feces	Di-gested
	Grams	Grams	Percent	Cal-ories	Cal-ories	Percent	Grams	Grams	Percent	Cal-ories	Cal-ories	Percent
1	17.36	2.64	84.8	2,553	234.5	90.8	17.48	1.32	92.4	2,612	81.0	96.9
2	19.66	2.85	85.5	2,896	270.5	90.7	19.79	1.39	93.0	2,962	90.6	96.9
3	17.04	2.43	85.7	2,506	231.5	90.8	17.19	1.39	91.9	2,568	86.6	96.6
4	14.76	1.74	88.2	2,166	182.3	91.6	14.83	1.19	92.0	2,211	73.2	96.7
5	15.64	2.23	85.7	2,297	192.3	91.6	15.83	1.12	92.9	2,362	69.6	97.1
6	17.59	2.52	85.7	2,588	238.7	90.8	17.71	1.17	93.4	2,647	79.2	97.0
Average	17.01	2.40	85.9	2,501	225.0	91.1	17.14	1.26	92.6	2,560	80.0	96.9

The interpretation of both experiments covered by this paper consists mainly of the comparison of groups of data representing the performance of individual rats on different dietary treatments, these comparisons being made by Love's modification of Student's method (Love (19)) of computation of the odds that differences between groups of data are not due to chance alone.

The rats which received the yellow corn ration made greater gains in live weight in all six comparisons than did the rats which received the white corn ration, the average gain of the rats on the yellow corn ration being 145.8 g, as compared with 120.8 g made by the rats on the white corn ration (table 1).

In five comparisons (table 2) a sixth comparison could not be made, since one ether extract was lost; the rats which received the yellow corn ration made more gain in energy than did their pair mates on the white corn ration, the average gains being 432.8 Calories with the yellow as compared with 364.7 Calories with the white corn ration. As to the distribution of this energy gain (table 3) the yellow corn ration was more efficient than the white in storing energy in the form of fat, and the white corn ration was more efficient than the yellow in storing energy in the form of protein.

In all six comparisons (table 4) the rats fed white corn digested more nitrogen and energy than did the rats fed yellow corn the average percentages digested being 85.9 percent nitrogen and 91.1 percent energy for the yellow corn, as compared with 92.6 percent nitrogen and 96.9 percent energy for the white corn ration.

It was noted that with each pair of rats the feces from the individual which received the white corn ration were decidedly darker in color than the feces from its pair mate. Obviously the dark color signified more complete digestion, but the reason for this difference was not apparent. In this connection it is significant that St. Julian and Heller (27) found that the digestibility of carbohydrate, protein, and fat are unaffected by a deficiency in vitamin A.

The grams of gain of nitrogen by the bodies of the rats was in favor of the yellow corn ration in 4 comparisons out of 6, 1 other being practically a tie, and 1 in favor of the white corn diet, but statistical criticism indicates that these differences were not certainly significant. There was also a greater percentage utilization of nitrogen in body gain by the rats on the yellow corn ration in 5 out of 6 comparisons. On an average 29.4 percent of the feed nitrogen of the yellow corn ration and 26.1 percent of that of the white corn ration were retained by the body, but the significance of this difference was found to be statistically questionable.

The grams gain of fat by the bodies of the rats was in favor of the yellow corn diet in 4 comparisons out of 5, averaging 28.9 g for the yellow and 23.1 g for the white corn diet, and was statistically significant. The difference in grams of fat per gram of body gain was not significant.

By way of recapitulation, therefore, the yellow corn ration was superior to the white in (1) palatability, (2) gain in weight, (3) gain in energy, and (4) the proportion of the energy gained which was present as fat. On the other hand the white corn ration was superior to the yellow in (1) digestibility of food nitrogen and (2) digestibility of food energy.

No figures were available for the energy utilization, since the urine was not collected.

From this preliminary experiment on yellow and white corn of unknown origin it is clear that the yellow corn used was in important respects superior to the white corn. In this relation the attention naturally directs itself to the difference in vitamin A content, but there is no logical reason for concluding that the observed differences in nutritive value were due solely to this obvious factor—among the many possible differences.

A SECOND EXPERIMENT

A second experiment was conducted, in which corn of known varieties was used. The required quantities of Reid Yellow Dent and Boone County White corn were obtained from a seed merchant in Richmond, Va. After grinding, the meal from the Reid Yellow Dent corn was quite yellow, and that from the Boone County White was "dead white." The rations were:

	Percent		Percent
Yellow corn	70.00	White corn	70.00
Crisco	10.18	Crisco	10.00
Cornstarch	1.59	Cornstarch	2.00
NaCl	1.00	NaCl	1.00
Osborne and Mendel salts	4.00	Osborne and Mendel salts	4.00
Casein (vitamin-A-free)	12.23	Casein (vitamin-A-free)	12.00
Irradiated yeast	1.00	Irradiated yeast	1.00
	100.00		100.00

The casein was a commercial vitamin-free preparation, and the yeast was a dry product of Fleischmann's manufacture containing 12 to 15 Sherman units each of vitamins B and G per gram. This yeast was irradiated and had a potency of 25 D, or 333 Steenbock units, per gram.

The yellow corn ration contained 4,521 calories per gram and 2.814 percent N (17.59 percent protein); and the white corn ration contained 4,528 calories per gram and 2.824 percent N (17.65 percent protein).

Ten pairs of albino rats were used, six pairs of females and four pairs of males. Their ages ranged from 24 to 28 days, and their initial weight from 38.99 to 48.86 g. The members of each pair were of the same litter and sex, and were within 3 g of the same weight. They were fed individually, one rat of each pair receiving the yellow-corn ration and the other the white-corn ration.

During a discussion of methods of feed allotment two procedures other than the paired method were temporarily employed. Thus, during the first 18 days the rats were fed by pairs, in direct proportion to their body weight for the day. During the next 8 days the feed allotment was based on the two-thirds power of a continuing 5-day average weight. Thereafter the regular paired feeding method was followed.

After the paired feeding method was adopted, the quantity of feed given per day was increased until there was refusal of feed by one individual of each pair. At the same time the feeding was so conducted, as to quantities assigned, that the total intake of the rats of each pair was equalized as soon as feasible. Nine of the pairs of rats were fed for 103 to 107 days, and the remaining pair (which were replacements) were fed for 78 days.

The feeding method, and the treatment of the metabolic products, were the same as explained, in some detail by Swift, Kahlenberg, Voris, and Forbes (34). Thus the metabolizable energy was computed as the gross energy of the food minus the energy of the urine and feces. Losses of carbon and energy occurring during the process of drying the urine were computed from the loss of nitrogen, on the basis of the assumption that the relationship between the energy and the carbon lost was as in urea.

The total heat production was determined indirectly by a difference procedure, as follows: A control group of rats, representative of the

rats to be fed, was killed at the beginning of the experiment. The alimentary contents were discarded and the bodies were analyzed for nitrogen, fat, and energy. At the end of the feeding experiment all rats were subjected to the same treatment as that given the control group. The body gains of nitrogen, fat, and energy made by the rats which were fed, therefore, were computed as the constituents of these rats minus those of the controls; and the heat production of the rats which were fed was computed as the gross energy of the food minus the energy of the excreta and of the body gain.

There was some coprophagy by rats on both diets. Watching revealed the fact that they would reach back and secure the feces pellets before they had a chance to fall through the screen false bottom of the cage, thus availing themselves of the benefit of the synthetic activities of the organisms of the alimentary tract.

The more important data resulting from this experiment are given in tables 5 to 10.

TABLE 5.—Gain in live weight¹ of rats fed yellow corn ration and white corn ration as related to dry matter of food

Pair no	Sex	Rats fed yellow corn			Rats fed white corn		
		Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in live weight	Ratio of gain to dry matter of food
		Gms	Gms	Percent	Gms	Gms	Percent
1.-----	♂	691.2	160.61	23.2	692.0	136.06	19.7
2.-----	♂	561.1	147.24	26.2	562.3	136.60	24.3
3.-----	♂	621.4	116.21	18.7	622.6	112.39	18.1
4.-----	♂	667.8	124.18	18.6	669.2	136.46	20.4
5.-----	♂	787.6	170.06	21.6	789.3	150.05	19.0
6.-----	♂	672.8	126.77	18.8	674.3	119.22	17.7
7.-----	♂	687.0	126.05	18.3	688.5	118.20	17.2
8.-----	♂	639.3	120.80	18.9	640.7	106.94	16.7
9.-----	♂	650.5	117.80	18.1	651.9	109.58	16.8
10.-----	♂	441.4	125.20	28.4	442.4	100.50	22.7
Average....		642.0	133.49	21.1	643.3	122.60	19.3

¹ Contents of alimentary tract removed

TABLE 6.—Energy of body gain of rats fed yellow corn ration and white corn ration as related to energy and to metabolizable energy of food

Pair no.	Rats fed yellow corn					Rats fed white corn					Body gain of energy of rats fed white corn as percentage of gain of pair mates
	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	
		Total	Percentage of food energy				Total	Percentage of food energy			
Calo-ries	Calo-ries	Per-cent	Calo-ries	Per-cent	Calo-ries	Calo-ries	Per-cent	Calo-ries	Per-cent	Per-cent	
1	3,450	388.3	11.3	3,040.6	12.8	3,452	401.7	11.6	3,045.3	13.2	103.5
2	2,801	422.7	15.1	2,437.3	17.3	2,805	403.1	14.4	2,452.2	16.4	95.4
3	3,102	333.0	10.7	2,717.1	12.3	3,106	370.8	11.9	2,722.6	13.6	111.4
4	3,333	375.6	11.3	2,911.2	12.9	3,338	451.3	13.5	2,937.8	15.4	120.2
5	3,931	462.4	11.8	3,425.6	13.5	3,938	573.1	14.6	3,463.0	16.5	123.9
6	3,359	340.5	10.1	2,925.2	11.6	3,364	483.3	14.4	2,980.6	16.3	141.9
7	3,429	370.0	10.8	2,983.5	12.4	3,434	318.7	9.3	3,019.9	10.6	86.1
8	3,191	351.4	11.0	2,822.5	12.4	3,196	287.7	9.0	2,843.1	10.1	81.9
9	3,247	287.2	8.8	2,872.4	10.0	3,252	364.2	11.2	2,869.0	12.7	126.8
10	2,204	314.9	14.3	1,952.1	16.1	2,207	246.2	11.2	1,952.6	12.6	78.2
Average...	3,205	364.6	11.5	2,808.7	13.1	3,209	390.0	12.1	2,826.6	13.7	106.9

TABLE 7.—Heat loss of rats fed yellow-corn ration and white-corn ration as related to energy of food

Pair no	Rats fed yellow corn			Rats fed white corn		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As percent- age of food energy		Total	As percent- age of food energy
	<i>Calories</i>	<i>Calories</i>	<i>Percent</i>	<i>Calories</i>	<i>Calories</i>	<i>Percent</i>
1	3,450	2,652.3	76.9	3,452	2,463.6	76.6
2	2,801	2,014.6	71.9	2,805	2,049.1	73.1
3	3,102	2,384.1	76.9	3,106	2,351.8	75.7
4	3,333	2,535.6	76.1	3,338	2,486.5	74.5
5	3,931	2,963.2	75.4	3,938	2,889.9	73.4
6	3,359	2,584.7	76.9	3,364	2,477.3	73.6
7	3,429	2,613.5	76.2	3,434	2,701.2	78.7
8	3,191	2,471.1	77.4	3,196	2,555.4	80.0
9	3,247	2,585.2	79.6	3,252	2,504.8	77.0
10	2,204	1,637.2	74.3	2,207	1,706.4	77.3
Average	3,205	2,444.2	76.2	3,209	2,436.6	76.0

TABLE 8.—Nitrogen of body gain of rats fed yellow-corn ration and white-corn ration as related to fat and energy of body gain and to nitrogen of food

RATS FED YELLOW CORN

Pair no	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>	<i>Percent</i>	<i>Percent</i>	<i>Grams</i>	<i>Percent</i>
1	5.79	19.3	3.3	398.3	47.2	52.8	21.48	27.0
2	5.37	25.6	4.8	422.7	56.9	43.1	17.43	30.8
3	3.99	20.9	5.2	333.0	59.7	40.3	19.31	20.7
4	4.21	24.7	5.9	375.6	62.0	38.0	20.75	20.3
5	5.93	27.2	4.6	462.4	55.6	44.4	24.47	24.2
6	4.39	19.6	4.5	340.5	54.6	45.4	20.91	21.0
7	4.28	23.8	5.6	370.0	60.3	39.7	21.34	20.1
8	3.90	22.8	5.8	351.4	60.8	39.2	19.86	19.6
9	4.09	15.3	3.7	287.2	50.6	49.4	20.21	20.2
10	4.53	16.4	3.6	314.9	49.5	50.5	13.72	23.0
Average	4.65	21.0	4.7	364.6	55.7	44.3	19.95	23.7

RATS FED WHITE CORN

1	4.83	24.9	5.2	401.7	57.7	42.3	21.53	22.4
2	5.13	24.0	4.7	403.1	56.1	43.9	17.49	20.3
3	3.73	26.1	7.0	370.8	66.4	33.6	19.37	19.3
4	4.37	32.0	7.3	451.3	67.3	32.7	20.82	21.0
5	4.45	44.3	10.0	573.1	73.2	26.8	24.56	18.1
6	3.38	38.7	11.4	463.3	75.4	24.6	20.08	16.1
7	4.02	19.2	4.8	318.7	56.4	43.6	21.42	18.8
8	3.72	16.6	4.5	287.7	54.3	45.7	19.93	18.7
9	3.46	25.3	7.3	364.2	65.3	34.7	20.28	17.1
10	3.69	12.3	3.3	246.2	47.3	52.7	13.76	26.8
Average	4.08	26.3	6.6	390.0	61.9	38.1	20.01	20.8

TABLE 9.—*Urinary carbon of rats fed yellow-corn ration and white-corn ration as related to urinary nitrogen*

Pair no	Rats fed yellow corn			Rats fed white corn		
	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio
	Grams	Grams		Grams	Grams	
1	11.34	12.89	0.9	12.15	13.88	0.9
2	9.72	9.73	1.0	9.90	10.10	1.0
3	10.75	12.43	.9	11.42	12.74	.9
4	10.20	13.60	.8	11.78	13.54	.9
5	13.32	14.43	.9	14.01	16.00	.9
6	11.70	13.72	.9	10.58	14.33	.7
7	12.37	13.13	.9	12.58	14.15	.9
8	10.76	13.25	.8	11.65	13.22	.9
9	10.90	13.34	.8	11.77	13.14	.9
10	6.70	7.59	.9	7.99	7.56	1.1
Average	10.78	12.41	.9	11.38	12.87	.9

TABLE 10.—*Digestibility of protein and energy-producing nutriment of yellow corn ration and white-corn ration when fed to rats*

Pair no	Rats fed yellow corn						Rats fed white corn					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Di-gested	Food	Feces	Di-gested	Food	Feces	Di-gested	Food	Feces	Di-gested
	Grams	Grams	Percent	Calo-ries	Calo-ries	Percent	Grams	Grams	Percent	Calo-ries	Calo-ries	Percent
1	21.48	2.09	90.3	3,450	282.2	91.8	21.53	2.03	90.6	3,452	273.8	92.1
2	17.43	1.93	88.9	2,801	261.9	90.6	17.49	1.88	89.3	2,805	248.4	91.1
3	19.31	1.98	89.7	3,102	264.8	91.5	19.37	2.14	89.0	3,106	258.6	91.7
4	20.75	2.18	89.5	3,333	306.5	90.8	20.82	2.11	89.9	3,338	271.5	91.9
5	24.47	2.58	89.5	3,931	363.1	90.8	24.56	2.56	89.6	3,938	323.0	91.8
6	20.91	2.04	90.2	3,359	306.2	90.9	20.98	2.22	89.4	3,364	283.1	91.6
7	21.34	2.04	87.6	3,429	312.2	90.9	21.42	1.88	91.2	3,434	279.2	91.9
8	19.86	1.70	91.4	3,191	253.4	92.1	19.93	1.66	91.7	3,196	222.0	93.1
9	20.21	1.88	90.7	3,247	256.5	92.1	20.28	2.32	88.6	3,252	235.4	92.1
10	13.72	1.11	91.9	2,204	178.3	91.9	13.76	1.25	90.9	2,207	170.8	92.3
Av.	19.95	2.01	90.0	3,205	278.5	91.3	20.01	2.01	90.0	3,209	258.6	92.0

During the entire feeding period there were 308 refusals of feed, 195 by the rats on the white-corn ration and 113 by the rats on the yellow-corn ration. On the basis of these numbers of refusals, without taking into account the quantities of feed refused, the deviation in the 195 refusals is 41 from the normal—154. This is 4.7 times the standard deviation, 8.77, and is therefore significant. This shows that the white corn was definitely the less palatable. It therefore served to determine the intake of both rations.

The data in table 5 shows that, although both members of each pair consumed essentially the same quantity of food, those rats that received the yellow-corn ration gained more weight than did their pair mates in 9 out of 10 comparisons, and the difference was significant. On the basis of the number of differences in weekly gain, which was in favor of the yellow-corn ration, the results are still more definitely significant.

The gain in live weight in relation to dry matter of food necessarily leads to conclusions of the same significance, since both rats of a pair

consumed the same quantity of food. The growth curves representing the gains in weight of the rats are to be found in figure 1, A.

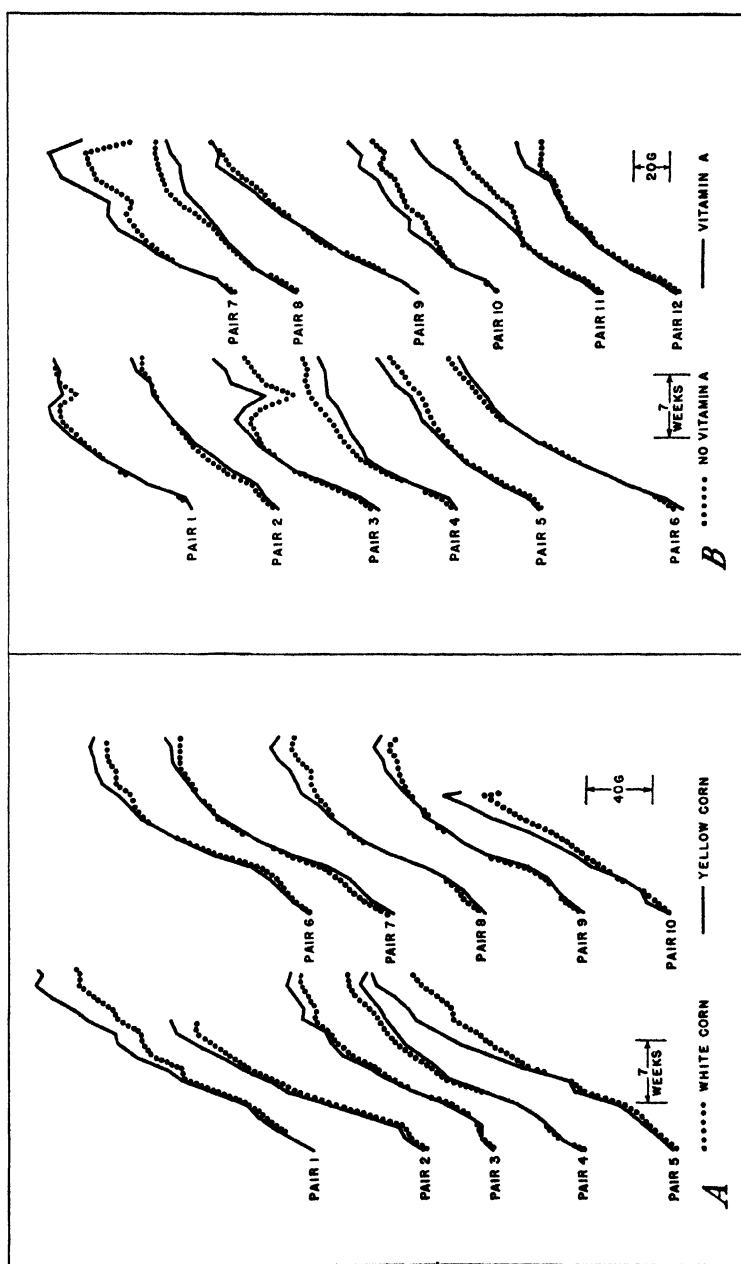


FIGURE 1.—Comparative growth of rats which received: (A) Equicaloric quantities of diets containing white and yellow corn; (B) equicaloric quantities of a complete diet and of a diet deficient in vitamin A.

In table 8 it is shown that the rats on the yellow-corn diet exceeded their pair mates on the white-corn diet in grams of nitrogen gained,

percentage of food nitrogen retained, and percentage of the energy gain which was present as protein; but it is shown in table 6 that there was no corresponding superiority of the rats fed yellow corn either in amount of energy gained or in efficiency of utilization of metabolizable energy; and in table 8 it is shown that the difference between the two groups in gains in fat and energy were indeterminate. Table 8 shows, however, that with the rats on the white-corn diet a larger proportion of the energy gained was present as fat; and there is somewhat significant evidence that there was more fat gained per gram of nitrogen gained than with the rats on the yellow-corn diet. The greater gain in live weight of the rats on the yellow-corn diet, therefore, consisted of protein and water.

Table 7 shows that there was no significant difference between the two groups as to heat loss.

Table 9 shows that with the rats fed white corn there was somewhat significant evidence of greater outgo of carbon and nitrogen in the urine, but the ratio of carbon to nitrogen in the urine of the two groups was not significantly different.

A difference in the digestibility of the two rations is shown in table 10, in that the energy-producing nutrients of the white-corn diet were slightly but definitely more digestible than were the energy-producing nutrients of the yellow-corn diet; but there was no significant difference in the digestibility of the protein of the two diets.

The ratios of carbon to nitrogen in the urines (table 9) varied between 0.8 and 1.0 for the yellow-corn ration, and between 0.7 and 1.1 for the white-corn ration, the average being 0.9 in both cases. With a low protein ration Swift, Kahlenberg, Voris, and Forbes (34) found, in experiments with rats, carbon to nitrogen ratios between 1.6 and 2; and in experiments with steers on a low-protein ration Forbes et al. (7) found a urinary carbon to nitrogen ratio as high as 4—these differences apparently being determined, at least in the main, by the plane of protein intake.

No effort was made to deplete the rats as to vitamin A previous to the experiment, and the failure of the rats which received the white-corn diet to manifest xerophthalmia indicates either that they contained a large initial store of this vitamin or that the white-corn diet contained a considerable quantity of this nutrient.

By way of recapitulation it may be said that in this second experiment the yellow-corn diet was shown to be definitely superior to the white-corn diet in the following respects: (1) Palatability, (2) gain in live weight, (3) grams of nitrogen retained, (4) percentage of the energy gained as protein, and (5) percentage of food nitrogen stored. The greater gain in live weight of the rats fed yellow corn consisted of protein and water.

The white-corn diet definitely excelled or exceeded the yellow-corn diet in the following respects: (1) Digestibility of the food energy, (2) percentage of food energy gained as fat, (3) fat gained per gram of nitrogen gained, (4) elimination of carbon in the urine, and (5) elimination of nitrogen in the urine.

The evidence failed to reveal a definite difference in the response of the animals to the two diets in the following respects: (1) Energy of body gain, (2) percentage of metabolizable energy stored, (3) grams of fat gained, (4) heat loss, (5) digestibility of food nitrogen, and (6) the carbon to nitrogen ratio in the urine.

Considering the two experiments together—the yellow-corn ration excelled the white-corn ration in (1) palatability and (2) gain in body weight; while the white-corn ration excelled in digestibility of food energy. In other respects the two experiments—insofar as the same observations were made—were not in definitely significant agreement; but it must be borne in mind that the first experiment covered only a part of the ground covered by the second.

In the interpretation of these results—since they involved a comparison of rations which differed only in the kind of corn—it is necessary to consider the possible influence of the method of food assignment. Thus, the rats which received yellow corn made the larger growth from the same food intake and therefore probably had the larger maintenance requirement—which is mainly for nonnitrogenous nutriment. The rats fed yellow corn, therefore, probably had left available for body increase (after maintenance requirements were satisfied) a smaller quantity and proportion of energy and a larger proportion of nitrogen than did the rats fed white corn. This fact, however, was without observable effect in the results obtained.

Finally, it is necessary to bear in mind the fact that whereas white and yellow corn are well known to differ in vitamin A content, the extent of this difference is far from constant; and it is not known that white and yellow corn differ only in vitamin A content. It must be understood, therefore, that even if it were an established fact (which it is not) that the vitamin A content of corn varies directly as the quantity of yellow-colored substance, there may still be other differences in nutritive value, possibly following other laws of distribution. The color of corn, therefore, while ordinarily indicative of nutritive value, cannot be considered positively significant in this respect.

THE EFFECTS OF VITAMIN A DEFICIENCY

Among the vitamins no other one has been so definitely associated with the phenomenon of growth as vitamin A. The details of this relationship, therefore, are of especial interest, and have been investigated in a growth, metabolism, and body analysis experiment with albino rats as subjects. The procedure as used was the same as that employed by Swift, Kahlenberg, Voris, and Forbes (34).

A somewhat similar experiment was conducted by Sampson and Korenchevsky (28), who studied the influence of vitamin A deficiency with 63 rats in an experiment in which results of paired and ad libitum feeding were compared. With the performance of the rats fed a complete diet, ad libitum, as the basis of comparison they concluded that two-fifths of the deficiency in the gain in weight of the A-deficient rats was due to deficient food intake, while three-fifths seemed to be due to lack of an anabolic principle; but in relation to fat deposition this relation was reversed—three-fifths of the deficiency being due to decreased food intake and two-fifths to lack of an anabolic principle.

Other papers relating to the effects of vitamin A deficiency on food utilization are reviewed in connection with the foregoing study of the present series.

In consideration of the storage capacity of the animal body for vitamin A the rats used in the present experiment were first subjected to a preparatory depletion of their stores of vitamin A, after which

they were divided into two groups composed of pairs of rats of the same litter, and matched as to sex and weight. One of these groups received a complete basal diet, and the other received a diet differing from the basal one only in that it was deficient in vitamin A. The experimental feeding covered a period of 84 days.

In the management of this experiment important details were the rate of preliminary depletion of the rats as to vitamin A stored within their bodies, and the rate of administration of carotene to the rats on the low-A diet.

Since the conditions of the experiment required that the vitamin-A-deficient rats be kept in a superficially normal condition—at least that they consume enough food to produce growth—and that this would be impossible in the presence of the profound disorganization of fully developed vitamin A deficiency, it was determined to maintain the deficient rats in only moderate vitamin A depletion, and to terminate the experiment as soon as the avitaminosis had become general and acute in the deficient group:

The growth curves (figure 1, *B*) show that the vitamin A deficiency became effective to limit growth at different times with the 12 pairs of rats, and that in general no such influence was apparent during a large part of the experimental period. This resulted mainly from the fact that the well-known characteristic effect of vitamin A deficiency on food intake, and therefore the main effect on growth, was excluded by the maintenance of equicaloric food consumption by pair mates.

The average daily gain in weight of the vitamin-A-deficient rats was 0.96g—which signifies that during the greater part of the experiment the shortage of vitamin A was indeed slight; but a high-grade deficiency would have made it impossible to carry out the plan of the experiment and to maintain equicaloric food intake with both groups of rats without disqualifying hardship to the rats which received the complete diet.

It must be understood, therefore, that the results obtained are representatives only of the particular conditions existing, and that they do not represent long-continued, acute vitamin A deficiency.

The main components of the basal diet were:

	Percent
Casein.....	18
Osborne and Mendel salt mixture	4
"Amidex" dextrin.....	68
Crisco.....	10
	<hr/> 100

In addition, a yeast mixture was fed to both groups of rats, to supply vitamins B, D, and G; and carotene was given regularly to one group, as a source of vitamin A, and to the other group in smaller quantity and only as definite symptoms of vitamin A deficiency were manifest.

The yeast mixture was composed of 57.6 g. of irradiated yeast and 950.4 g. of dried brewer's yeast; and of this a 0.42-g. portion was fed daily to each rat. This supplied 8 Steenbock units of vitamin D, and 5 Sherman units of vitamins B and G. This amount of vitamin D is considered sufficient for normal calcification.

The rats on the complete diet were given 2 International units of vitamin A per day. This was administered as 0.1 cc of a solution of carotene prepared from a carotene-corn-oil solution of a potency of 10,000 International units per gram. The solution as administered was prepared by diluting 0.8 g. of the carotene-corn oil solution with petroleum ether to make 400 cc.

The solution of carotene for the rats on the deficient diet was one-sixteenth the concentration of the solution for the rats on the complete diet, the daily portion of one twenty-fifth cubic centimeter on which they were started containing 0.05 International unit. During the course of the experiment, however, as these rats appeared to require increased amounts of carotene, a solution of four times the concentration stated was prepared, containing 0.1 International unit per one-fiftieth cubic centimeter. Of this more concentrated solution quantities of one-fiftieth cubic centimeter per day or more were administered as required. The more dilute solutions evidently deteriorated, since, during the progress of the experiment, an increasing volume of solution was necessary to maintain a fairly satisfactory food consumption. Nothing was added to this solution to prevent oxidation of the fat in which the carotene was contained. Mattill (20) has suggested hydroquinone, among other substances, as an antioxidant for preserving such solutions.

The moderate preliminary depletion of the experimental subjects as to vitamin A was accomplished by feeding the mother rats the vitamin-A-free diet and 8 percent of the yeast mixture mentioned above, from the time the young rats were 2 weeks old until they were weaned at the age of 3 weeks. Foods rich in vitamin A, such as carrots, were withheld during the last 2 weeks of the period of gestation, and milk was fed to the mother rats only during the last week of gestation and thereafter until the young rats were weaned.

In the latter part of the feeding period an attempt was made to detect evidence of the lack of vitamin A by microscopic examination of the vaginal smears, by the technic of Long and Evans (18). This observation revealed evidence of the vitamin A deficiency in a number of the rats before it was manifested by any observable external appearance.

The vitamin A deficiency appeared among the 12 rats on the deficient diet after 34 to 55 days. Carotene was then administered from time to time as required to maintain the rats in quasi-normality—that is, on the border line between health and disease, but eating a fair quantity of food.

After 84 days on the experiment the rats which had received carotene regularly were in good condition and showed no signs of lack of vitamin A, either by outward appearance or by the condition of the vaginal smears; but all of their pair mates, which had received the deficient diet, manifested, in various degrees of severity, the well known symptoms of vitamin A deficiency, including the characteristic histology of the vaginal exudate, xerophthalmia, cessation of growth, and lack of normal muscular control in locomotion.

At the end of the feeding experiment all 24 rats were killed, and the bodies, the excreta, and the diets were subjected to chemical analysis. The resulting data are shown in tables 11 to 16.

TABLE 11.—*Gain in live¹ weight of rats fed vitamin-A-supplemented or vitamin-A-deficient diets as related to dry matter of food*

Pair no.	Sex	Rats fed vitamin-A-supplemented diet			Rats fed vitamin-A-deficient diet		
		Food eaten (dry matter)	Gain in empty live weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in empty live weight	Ratio of gain to dry matter of food
		Grams	Grams	Percent	Grams	Grams	Percent
1.	♀	413.5	77.17	18.7	413.5	77.57	18.8
2.	♀	401.8	80.00	19.9	401.8	71.84	17.9
3.	♂	407.9	91.00	22.3	406.9	68.74	16.9
4.	♀	403.0	73.58	18.3	403.0	82.93	20.6
5.	♀	395.3	90.14	22.8	395.3	83.70	21.2
6.	♂	492.8	123.10	25.0	492.8	123.04	25.0
7.	♂	457.7	79.65	17.4	454.2	50.51	11.1
8.	♀	467.7	72.89	15.6	467.7	77.75	16.6
9.	♂	519.9	112.57	21.7	519.9	111.93	21.5
10.	♀	438.4	81.09	18.5	438.4	60.71	15.2
11.	♂	479.9	102.38	21.3	479.9	79.22	16.5
12.	♀	519.2	88.47	17.0	519.2	75.08	14.5
Average ..		449.8	89.34	19.9	449.4	80.75	18.0

¹ Contents of alimentary tract removedTABLE 12.—*Energy of body gain of rats on vitamin-A-supplemented or vitamin-A-deficient diets as related to energy and to metabolizable energy of food*

Pair no	Rats fed vitamin-A-supplemented diet					Rats fed vitamin-A-deficient diet					Body gain of energy of vitamin-A-free rats as percent-age of gain of pair mate
	Food energy	Body gain		Metab-olizable energy	Body gain as percent-age of metab-olizable energy	Food energy	Body gain		Metab-olizable energy	Body gain as percent-age of metab-olizable energy	
		Total	As per-cent-age of food energy				Total	Per-cent-of food energy			
	Calo-ries	Calo-ries	Per-cent	Calories	Percent	Calo-ries	Calo-ries	Per-cent	Calories	Percent	Percent
1	1,848	239.3	12.9	1,687.3	14.2	1,848	252.8	13.7	1,690.4	15.0	105.6
2	1,796	210.6	11.7	1,621.7	13.0	1,796	245.8	13.7	1,619.2	15.2	116.7
3	1,823	232.6	12.8	1,685.8	13.8	1,818	175.3	9.6	1,651.2	10.6	75.4
4	1,801	233.3	13.0	1,658.9	14.1	1,801	275.7	15.3	1,652.0	16.7	118.2
5	1,767	291.3	15.5	1,658.1	17.6	1,767	284.9	16.1	1,619.1	17.6	97.8
6	2,201	320.5	14.6	2,025.9	15.8	2,201	336.9	15.3	2,023.5	16.6	105.1
7	2,045	176.6	8.6	1,879.1	9.4	2,029	76.5	3.8	1,838.7	4.2	43.3
8	2,089	214.5	10.3	1,910.7	11.2	2,089	227.0	10.9	1,918.5	11.8	105.8
9	2,322	295.5	12.7	2,149.9	13.7	2,322	267.6	11.5	2,137.4	12.5	90.6
10	1,959	197.9	10.1	1,799.2	11.0	1,959	167.1	8.5	1,782.3	9.4	84.4
11	2,143	250.6	11.7	1,985.0	12.6	2,143	176.8	8.3	1,952.5	9.1	70.6
12	2,319	244.9	10.6	2,128.4	11.5	2,319	189.5	8.2	2,133.5	8.9	77.4
Average	2,009	242.3	12.1	1,849.2	13.2	2,008	223.0	11.2	1,834.9	12.3	80.9

TABLE 13.—Heat loss of rats on vitamin-A-supplemented or vitamin-A-deficient diets as related to energy of food

Pair no.	Rats fed vitamin-A-supplemented diet			Rats fed vitamin-A-deficient diet		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As per-centage of feed energy		Total	As per-centage of feed energy
	<i>Calories</i>	<i>Calories</i>	<i>Percent</i>	<i>Calories</i>	<i>Calories</i>	<i>Percent</i>
1	1,848	1,448 0	78.4	1,848	1,437 6	77.8
2	1,796	1,411.1	78.6	1,796	1,373.4	76.5
3	1,823	1,453.2	79.7	1,818	1,475.9	81.2
4	1,801	1,425.6	79.2	1,801	1,376.3	76.4
5	1,767	1,366.8	77.4	1,767	1,334.2	75.5
6	2,201	1,705.4	77.5	2,201	1,686.6	76.6
7	2,045	1,702.5	83.3	2,029	1,762.2	86.9
8	2,089	1,696.2	81.2	2,089	1,691.5	81.0
9	2,322	1,854.4	79.9	2,322	1,869.8	80.5
10	1,959	1,601.3	81.7	1,959	1,615.2	82.5
11	2,143	1,734.4	80.9	2,143	1,775.7	82.9
12	2,319	1,883.5	81.2	2,319	1,944.0	83.8
Average	2,009	1,666.9	79.9	2,008	1,611.9	80.1

TABLE 14.—Nitrogen of body gain of rats fed vitamin-A-supplemented or vitamin-A-deficient diets as related to fat and energy of body gain and to nitrogen of food

RATS FED VITAMIN-A-SUPPLEMENTED DIET

Pair no	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram of nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>	<i>Percent</i>	<i>Percent</i>	<i>Grams</i>	<i>Percent</i>
1	2.70	15.7	5.8	239.3	60.9	39.1	13.95	19.4
2	2.43	13.6	5.6	210.6	59.7	40.3	13.62	17.8
3	2.91	14.4	4.9	232.6	57.2	42.8	13.79	21.1
4	2.19	17.3	7.9	233.3	68.4	31.6	13.65	16.0
5	2.76	21.5	7.8	201.3	68.8	31.2	13.43	20.6
6	3.99	19.8	5.0	320.5	57.9	42.1	16.21	24.6
7	2.98	8.3	2.8	176.6	43.8	56.2	15.21	19.6
8	2.32	14.4	6.2	214.5	62.4	37.6	15.50	15.0
9	3.53	18.6	5.3	295.5	58.0	42.0	16.98	20.8
10	2.82	11.2	4.0	197.9	52.2	47.8	14.66	19.2
11	3.31	14.1	4.3	250.6	51.5	48.5	15.84	20.9
12	2.83	16.2	5.7	244.9	61.4	38.6	16.97	16.7
Average	2.90	15.4	5.4	242.3	58.5	41.5	14.98	19.3

RATS FED VITAMIN-A DEFICIENT DIET

1	2.53	17.5	6.9	252.8	64.1	35.9	13.95	18.1
2	2.13	19.2	9.0	245.8	71.6	28.4	13.62	15.6
3	2.47	9.8	4.0	175.3	51.3	48.7	13.76	18.0
4	2.55	20.2	7.9	275.7	68.6	31.4	13.65	18.7
5	2.36	21.7	9.2	284.9	71.0	29.0	13.43	17.6
6	4.03	21.6	5.4	336.0	59.3	40.7	16.21	24.9
7	2.21	5.5	2.2	76.5	3.1	96.9	15.11	14.6
8	2.46	15.3	6.2	227.0	63.0	37.0	15.50	15.9
9	3.82	14.9	3.9	267.6	51.6	48.4	16.98	22.5
10	2.37	6.5	4.0	167.1	51.9	48.1	14.66	16.2
11	2.41	10.2	4.2	176.8	53.1	46.9	15.84	15.2
12	2.54	11.2	4.4	189.5	55.2	44.8	16.97	15.0
Average	2.66	14.3	5.4	223.0	55.3	44.7	14.97	17.7

TABLE 15.—*Urinary carbon of rats fed vitamin-A-supplemented or vitamin-A-deficient diets as related to urinary nitrogen*

Pair no.	Rats fed vitamin A			Rats fed vitamin-A-deficient diet		
	Urinary carbon	Urinary nitrogen	Carbon/nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon/nitrogen ratio
	Grams	Grams		Grams	Grams	
1.....	5.96	9.63	0.6	6.73	10.03	0.7
2.....	6.56	9.77	.7	8.18	10.41	.8
3.....	6.30	9.73	.6	7.03	9.87	.7
4.....	6.91	10.34	.7	7.10	10.07	.7
5.....	3.71	6.04	.6	6.23	10.36	.6
6.....	7.45	11.07	.7	7.84	10.80	.7
7.....	7.92	11.02	.7	10.03	11.13	.9
8.....	9.87	11.70	.8	7.11	11.48	.6
9.....	7.62	11.61	.7	8.05	11.50	.7
10.....	7.48	10.59	.7	9.91	10.62	.9
11.....	7.81	11.30	.7	10.60	11.60	.9
12.....	9.63	12.89	.7	8.56	13.08	.7
Average.....	7.27	10.47	.7	8.11	10.91	.7

TABLE 16.—*Digestibility of protein and energy-producing nutriment of diets containing or deficient in vitamin A, when fed to rats*

Pair no.	Rats fed vitamin-A-supplemented diet						Rats fed vitamin-A-deficient diet					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested	Food	Feces	Digested
	Grams	Grams	Percent	Calories	Calories	Percent	Grams	Grams	Percent	Calories	Calories	Percent
1.....	13.95	1.09	92.2	1,848	90.5	95.1	13.95	1.03	92.6	1,848	79.4	95.7
2.....	13.62	.92	93.2	1,796	96.8	94.6	13.62	.92	93.2	1,796	84.8	95.3
3.....	13.79	.82	94.1	1,823	63.3	96.5	13.76	1.03	92.5	1,818	87.4	95.2
4.....	13.65	.75	94.5	1,801	62.5	96.5	13.65	.90	93.4	1,801	68.1	96.2
5.....	13.43	.93	93.1	1,767	65.7	96.3	13.43	.88	93.4	1,767	74.5	95.8
6.....	16.21	1.08	93.3	2,201	89.3	95.9	16.21	1.10	93.2	2,201	89.6	95.9
7.....	15.21	.94	93.8	2,045	75.7	96.3	15.11	1.06	93.0	2,029	80.0	96.1
8.....	15.50	.94	93.9	2,089	69.2	96.7	15.50	1.13	92.7	2,089	85.8	95.9
9.....	16.98	1.04	93.9	2,322	82.9	96.4	16.98	1.05	93.8	2,322	90.5	96.1
10.....	14.66	.99	93.2	1,959	74.0	96.2	14.66	.86	94.1	1,959	67.7	96.5
11.....	15.84	.92	94.2	2,143	69.1	96.8	15.84	.96	93.9	2,143	73.9	96.6
12.....	16.97	1.00	94.1	2,319	81.3	96.5	16.97	1.24	92.7	2,319	86.0	96.3
Average.....	14.98	.95	93.6	2,009	76.7	96.2	14.97	1.01	93.2	2,008	80.6	96.0

In 8 of the 12 pairs of rats, those which received the complete diet made greater gains in live weight than did those which received the vitamin-A-deficient diet, though, in accord with the method of experimentation, both members of each pair consumed the same amount of food. The vitamin-A-deficient rats undoubtedly held back the food consumption and the growth of their pair mates by not consuming as much of the ration as the rats on the complete diet would have eaten. This is shown by the fact that the rats on the vitamin-A-deficient diet refused portions of their food a total of 466 times—as compared with 249 refusals by their pair mates. This is a statistically significant difference.

The growth curves are shown in figure 1, *B*. The average gains in live weight (table 11) of the rats on the complete and on the vitamin-A-deficient diets were 89.34 g and 80.75 g, respectively, this difference being statistically significant.

Table 12 shows that the two groups did not differ in a statistically significant manner with reference to (1) energy of body gain, (2) energy of body gain as a percentage of the food energy, or (3) body gain of energy as a percentage of the metabolizable energy. Similarly, table 13 shows that the heat production was essentially the same for the two groups of rats.

Table 14 shows that the nitrogen storage of the rats on the complete diet was significantly greater than that of their pair mates on the vitamin-A-deficient diet; but there was not a significant difference in the storage of fat, or of fat gained per gram of nitrogen gained, or of total energy gained, or of energy gained as fat or as protein.

The analysis of the urines (table 15) reveals as slightly significant the greater elimination of carbon by the rats on the vitamin-A-deficient diet, but the ratio of carbon to nitrogen in the urine did not differ significantly; nor did the digestibility (table 16) of either the nitrogen or the energy of the diet.

THE EFFECTS OF VITAMIN D DEFICIENCY

Among the various requirements of complete nutrition it is obvious that the different essential nutrients must differ in the nature and the directness of their relationship to the utilization of the protein and the energy of foods; and, in the light of the existing knowledge of the characteristic functions of vitamin D, as essential to the normal utilization of calcium and phosphorus, the relationship of this vitamin to protein and energy metabolism must appear to be indirect and secondary, rather than immediate and essential. However, this relationship may be none the less real for being incidental, and the object of the present experiment was to show the extent and something of the nature of the influence of vitamin D in the connection stated. Since the writers know of no other study of this vitamin made from the point of view of the present investigation, this experiment will be described without general review of the literature on vitamin D.

This experiment consisted of a comparison of two dietary regimens, by means of a growth-, metabolism-, and body-analysis study, on two groups of normal growing rats. One of the diets was known to cause rickets, the other was the same diet plus a vitamin D concentrate.

The rachitogenic diet selected for use in this experiment was Steenbock's diet no. 2965 (31), which consisted of yellow corn 76 percent, wheat gluten 20 percent, CaCO_3 3 percent, and NaCl 1 percent.

This diet has been considered by Bacharach, Allehorne, and Hazley (1) to be deficient in vitamin A, as well as in vitamin D; but in the study reported in the first section of this paper involving the particular lot of yellow corn used in the present experiment, no evidence of vitamin A deficiency was encountered.

Goldblat (10) observed irregular results from the feeding of vitamin-D-free rations containing much CaCO_3 , apparently due to a disturbance of the ratio of calcium to vitamin D, through the settling out of CaCO_3 from the ration. In the present experiment the diets were stored in 2-quart Mason jars, the contents of which were mixed whenever food was removed. The rats did not grow rapidly, but their performance was consistent in the manner of their indicating the presence or the absence of vitamin D.

In this experiment, as in the others of this series, the paired feeding method was used, the subjects being 12 pairs of weanling rats, 27 to 29 days of age, and weighing 42 to 60 g at the beginning of the study.

The individuals of each pair were of the same sex, age, and litter, and of approximately the same body weight. The general routine followed was as in the foregoing studies of this series.

Both rats of each pair received, throughout the experiment, the same quantity of the basal rachitogenic ration specified; but after the first 2 weeks one rat of each pair received in addition 1 vitamin D unit

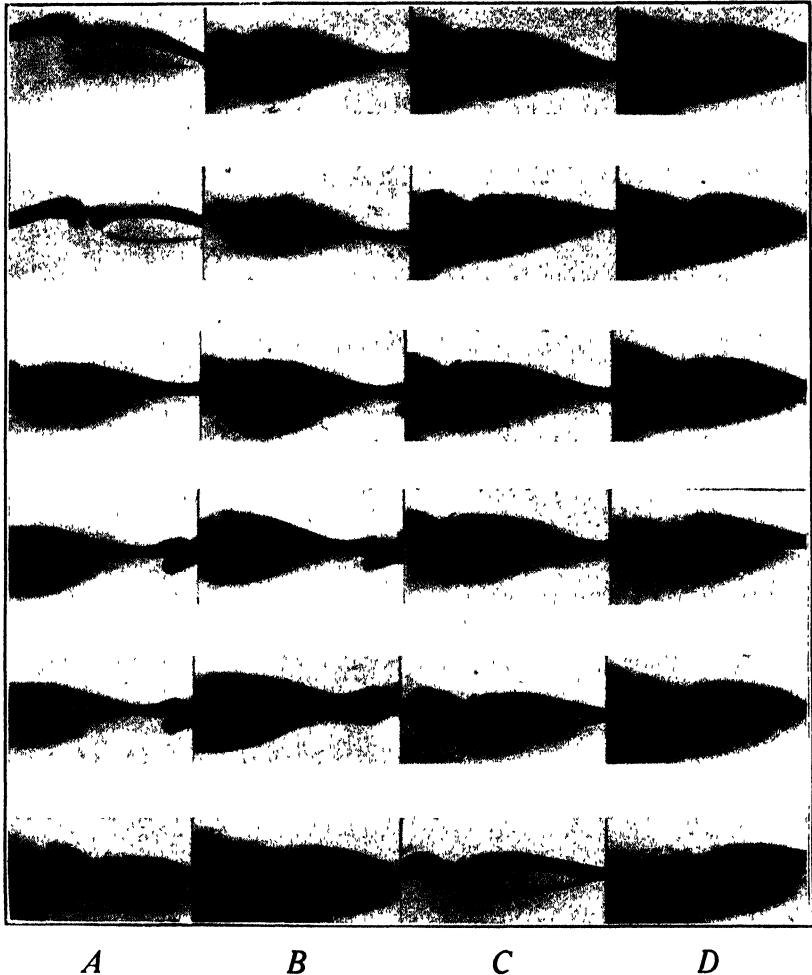


FIGURE 2—Photographs of leg bones of rats which received a complete diet (A and C) compared with others which received a vitamin D deficient diet (B and D). Pairs are arranged in order of number, and pair mates are placed side by side

(Steenbock) of viosterol (Squibb) each day. For 3 weeks the viosterol was fed in petroleum ether dropped on top of the food, the ether being assumed to have evaporated before the food was offered. Three weeks' experience, however, showed that the petroleum ether did not entirely evaporate, and that it unfavorably affected the palatability of the food. Thereafter the viosterol was fed in olive oil, and the rats which received no viosterol were given olive oil in the same

amounts as were their pair mates. The olive oil improved the appetite of the rats. The refusals of food to the time of beginning the use of olive oil were nearly alike in number for the two groups, but thereafter the number of refusals was significantly less with the rats which received the complete diet.

The feeding period lasted 10 weeks. All of the rats on the basal diet manifested symptoms of vitamin D deficiency, while those which received vitamin D were normal. X-ray photographs of one hind leg of each animal are shown in Figure 2. None of the animals showed any noticeable symptoms of vitamin A deficiency.

In the daily care of the rats the feed-cup holders were removed from the cages in the morning and were not returned until feeding time in the afternoon. This prevented the rats from depositing urine and feces in them.

As soon as the rats on the deficient diet developed noticeable signs of rickets, such as lagging with the hind legs, they and their pair mates were killed, and an X-ray photograph was taken of one hind leg of each individual. The bodies were then analyzed as in the foregoing studies, and the experimental data, given in tables 17 to 22 were statistically interpreted.

TABLE 17. - *Gain in live weight¹ of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to dry matter of feed*

Pair no	Sex	Rats fed vitamin-D-supplemented diet			Rats fed vitamin-D-deficient diet		
		Food eaten (dry-mat- ter)	Gain in empty live weight	Ratio of gain to dry matter of food	Food eaten (dry mat- ter)	Gain in empty live weight	Ratio of gain to dry matter of food
		Grams	Grams	Percent	Grams	Grams	Percent
1	♀	274 2	34 10	12 4	274 2	33 86	12 3
2	♀	312 6	45 58	14 6	312 6	33 30	10 7
3	♂	292 9	44 16	15 2	292 9	36 16	12 3
4	♂	326 2	38 44	11 8	326 2	37 31	11 4
5	♂	306 1	31 83	10 4	306 1	37 94	12 4
6	♂	379 1	39 95	10 5	377 0	39 21	10 4
7	♂	342 0	33 81	9 9	342 0	38 57	11 3
8	♂	244 6	14 72	6 0	244 6	21 11	8 6
9	♂	298 9	28 79	9 6	298 9	27 59	9 2
10	♂	293 8	30 24	10 3	293 8	26 69	9 1
11	♂	299 6	37 11	12 4	299 5	36 80	12 3
12	♂	370 7	50 97	13 7	370 1	38 59	10 4
Average.		311 7	35 83	11 4	311 5	33 93	10 9

¹ Contents of alimentary tract remove l.

TABLE 18.—*Energy of body gain of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to energy and to metabolizable energy of food*

Pair no.	Rats fed vitamin-D-supplemented diet					Rats fed vitamin-D-deficient diet					Body gain of energy of vitamin D free rats as percentage of gain of pair mate
	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	
		Total	As percentage of feed energy				Total	As percentage of feed energy			
	Calories	Calories	Percent	Percent	Percent	Calories	Calories	Percent	Calories	Percent	Percent
1	1,270	126 1	9 9	1,079.0	11 7	1,270	72 4	5 7	1,075 5	6 7	57 4
2	1,445	144 5	10 0	1,229 4	11 8	1,445	94 9	6 6	1,210 9	7 4	65 7
3	1,355	121.6	9 0	1,148.6	10 6	1,355	71 4	5 3	1,146 4	6 2	58 7
4	1,508	103 5	6 9	1,276 9	8 1	1,508	92 8	6 2	1,275 3	7 3	89 7
5	1,415	64 4	4 6	1,187 6	5 4	1,415	81 4	5 8	1,190 8	6 8	126 4
6	1,749	111 3	6 4	1,467 8	7 6	1,740	92 5	5 3	1,459 3	6 3	83 1
7	1,581	155 6	9 8	1,332 0	11 7	1,581	124 8	7 9	1,342 8	9 3	80 2
8	1,134	34.8	3 1	950 7	3.7	1,134	36 8	3 2	963 5	3 8	105 7
9	1,383	77 1	5 6	1,164 5	6 6	1,383	53 2	3 8	1,106 5	4 6	69 0
10	1,360	47 8	3 5	1,146 6	4 2	1,360	35 7	2 6	1,144 2	3 1	71 7
11	1,384	92 7	6 7	1,152 7	8 0	1,384	68 7	5 0	1,166 0	5 9	74 1
12	1,711	121 7	7 1	1,429 8	8 5	1,709	67.3	3 9	1,443 7	4 7	55 3
Average	1,441	100 1	6 9	1,213 8	8 2	1,440	71 3	5.1	1,215 4	6 0	74 3

TABLE 19.—*Heat loss of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to energy of food*

Pair no.	Rats fed vitamin-D-supplemented diet			Rats fed vitamin-D-deficient diet		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As percentage of feed energy		Total	As percentage of feed energy
	Calories	Calories	Percent	Calories	Calories	Percent
1	1,270	952 9	75 0	1,270	1,063 1	79 0
2	1,445	1,084 9	75 1	1,445	1,116.0	77 2
3	1,355	1,027.0	75 8	1,355	1,075 0	79 3
4	1,508	1,173 4	77 8	1,508	1,182 5	78 4
5	1,415	1,123 2	79 4	1,415	1,109 4	78 4
6	1,749	1,350 5	77 6	1,740	1,366 8	78 6
7	1,581	1,176 4	74 4	1,581	1,218.0	77 0
8	1,134	915 9	80 8	1,134	926 7	81 7
9	1,383	1,087 4	78.6	1,383	1,113 3	80.5
10	1,360	1,098 8	80 8	1,360	1,108.5	81 5
11	1,384	1,060.0	76 6	1,384	1,097 3	79 3
12	1,711	1,308 1	76.5	1,709	1,376.4	80 5
Average	1,441	1,113 7	77.4	1,440	1,141.1	79 3

TABLE 20.—*Nitrogen of body gain of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to fat and energy of body gain and to nitrogen of food*

RATS FED VITAMIN-D-SUPPLEMENTED DIET

Pair no.	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	Grams	Grams	Grams	Calories	Percent	Percent	Grams	Percent
1	1.30	8.5	6.5	126.1	64.6	35.4	11.52	11.3
2	1.69	8.9	5.3	144.5	56.7	43.3	13.15	12.9
3	1.68	6.7	4.0	121.6	51.4	48.6	12.32	13.6
4	1.59	4.9	3.1	103.5	44.0	56.0	13.73	11.6
5	1.46	1.3	.9	64.4	18.6	81.4	12.87	11.3
6	1.70	5.6	3.3	111.3	46.5	53.5	15.98	10.6
7	1.20	11.5	9.6	155.6	70.2	29.8	14.40	8.3
8	.86	.2	.2	34.8	5.5	94.5	10.27	8.4
9	1.36	2.5	1.8	77.1	28.7	71.3	12.57	10.8
10	1.35	.5	.3	47.8	8.6	91.4	12.35	10.9
11	1.63	4.0	2.5	92.7	39.1	60.9	12.61	12.9
12	2.06	5.2	2.5	121.7	39.2	60.8	15.62	13.2
Average	1.49	5.0	3.3	100.1	39.4	60.6	13.12	11.3

RATS FED VITAMIN-D-DEFICIENT DIET

1	1.66	2.0	1.2	72.4	25.8	74.2	11.52	14.4
2	1.48	4.8	3.2	94.9	45.8	54.2	13.15	11.3
3	1.65	1.6	1.0	71.4	20.6	79.4	12.32	13.4
4	1.71	3.8	2.2	92.8	38.4	61.6	13.73	12.5
5	1.87	2.0	1.1	81.4	22.2	77.8	12.87	14.5
6	1.86	3.5	1.9	92.5	34.4	65.6	15.89	11.7
7	1.70	6.5	3.8	124.8	49.4	50.6	14.40	11.8
8	1.20	—	—	36.8	—16.0	116.0	10.27	11.7
9	1.41	—	—	53.2	16.9	83.1	12.57	11.2
10	1.39	—1.0	—	35.7	—25.5	125.5	12.35	11.3
11	1.86	1.1	.6	68.7	13.7	86.3	12.61	14.8
12	1.91	.3	.2	67.3	4.2	95.8	15.59	12.3
Average	1.64	2.1	1.2	74.3	19.2	80.8	13.11	12.6

TABLE 21.—*Urinary carbon of rats fed vitamin-D-supplemented or vitamin-D-deficient diets as related to urinary nitrogen*

Pair no.	Rats fed vitamin-D-supplemented diet			Rats fed vitamin-D-deficient diet		
	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon nitrogen ratio
	Grams	Grams		Grams	Grams	
1	7.14	9.03	0.8	6.88	8.62	0.8
2	7.87	9.86	.8	7.70	9.75	.8
3	7.27	9.00	.8	7.16	9.17	.8
4	8.53	9.89	.9	8.46	10.18	.8
5	8.11	9.01	.9	7.85	9.46	.8
6	9.52	11.63	.8	9.24	11.76	.8
7	8.96	11.40	.8	8.35	10.36	.8
8	6.38	8.29	.8	6.15	7.85	.8
9	7.81	9.55	.8	7.51	9.57	.8
10	7.88	8.99	.9	7.91	9.38	.8
11	8.18	9.41	.9	7.36	9.11	.8
12	9.81	11.44	.9	9.90	11.17	.9
Average	8.12	9.79	.8	7.87	9.70	.8

TABLE 22.—*Digestibility of protein and energy-producing nutriment of diets containing or lacking vitamin D, when fed to rats*

Pair no	Rats fed vitamin-D-supplemented diet						Rats fed vitamin-D-deficient diet					
	Nitrogen			Energy			Nitrogen			Energy		
	Food	Feces	Dig- ested	Food	Feces	Dig- ested	Food	Feces	Dig- ested	Food	Feces	Dig- ested
	Grams	Grams	Per- cent	Calo- ries	Calo- ries	Per- cent	Grams	Grams	Per- cent	Calo- ries	Calo- ries	Per- cent
1	11 52	0 77	93 3	1,270	115 6	90 9	11 52	0 85	92 6	1,270	120 7	91 5
2	13 15	95	92 8	1,445	132 2	90 9	13 15	1 10	91 6	1,445	149 8	89 6
3	12 32	86	93 0	1,355	127 8	90 6	12 32	84	93 2	1,355	128 6	90 5
4	13 73	1 02	92 6	1,508	140 2	90 7	13 73	99	92 8	1,508	139 2	91 8
5	12 87	1 14	91 1	1,415	140 7	90 1	12 87	1 01	92 2	1,415	139 7	90 1
6	15 98	1 32	91 7	1,749	177 1	89 9	15 98	1 34	91 6	1,740	179 0	89 7
7	14 40	1 07	92 6	1,581	154 8	90 2	14 40	1 10	92 4	1,581	146 6	90 7
8	10 27	81	92 1	1,134	113 7	90 0	10 27	77	92 5	1,134	103 5	90 9
9	12 57	89	92 9	1,383	133 4	90 4	12 57	97	92 3	1,383	133 5	91 3
10	12 35	97	92 1	1,360	130 3	90 4	12 35	97	92 1	1,360	130 3	90 4
11	12 61	99	92 1	1,384	145 0	89 5	12 61	90	92 9	1,384	137 2	90 1
12	15 62	1 31	91 6	1,711	176 7	89 7	15 59	1 16	92 6	1,709	160 9	90 6
Average	13. 12	1 01	92 3	1,441	149 6	90 3	13 11	1 00	92 4	1,440	139 1	90 4

PRESENTATION OF RESULTS

Both animals of a pair received the same amount of the same diet (the weight of the viosterol administered being negligible) and the animals were weighed daily in the morning before feeding. The growth curves for the entire feeding period are shown in figure 3, A.

The gains in gross live weight revealed 6 greater gains by the vitamin D rats, and 6 by the rats on the vitamin D deficient diet; and the gains in empty body weight showed that in 9 cases of the 12 the vitamin D rats made larger gains than did their pair mates (table 17); but the gains were small, and on neither basis of comparison were there statistically significant differences between the results of the 2 treatments.

Although the rats showed no signs of lack of vitamin A, they grew but slowly. In view of the method of food apportionment employed, the rats on the rachitogenic diet undoubtedly held back their pair mates, in food consumption and in growth. The rats on the vitamin deficient diet refused a part of their food a total of 381 times as compared with 259 refusals for the rats on the supplemented diets. This difference was statistically significant.

The development of rickets is more pronounced, on a rachitogenic diet, when growth is more rapid than it was in this experiment. However, a comparison of the X-ray photographs of the rats, taken at the end of 10 weeks, showed a distinct effect of the vitamin deficiency. Those individuals which received viosterol regularly showed normal development of the bones, while all of those on the rachitogenic diet showed malformation as shown in figure 2.

On an average, for the 12 pairs, 94.04 percent (90.21 to 96.98) of the food nitrogen was recovered in the urine, the feces, and the body gains. The nitrogen not accounted for may be considered to represent shed hair, ammonia evaporated from the urine, and analytical error.

The rats on the complete diet gained significantly more fat and energy (table 20) and eliminated more carbon in the urine (table 21) than did the rats on the vitamin-D-deficient diet.

With the vitamin-D-deficient rats there was a higher percentage utilization of nitrogen for body gain (table 20), a higher percentage of

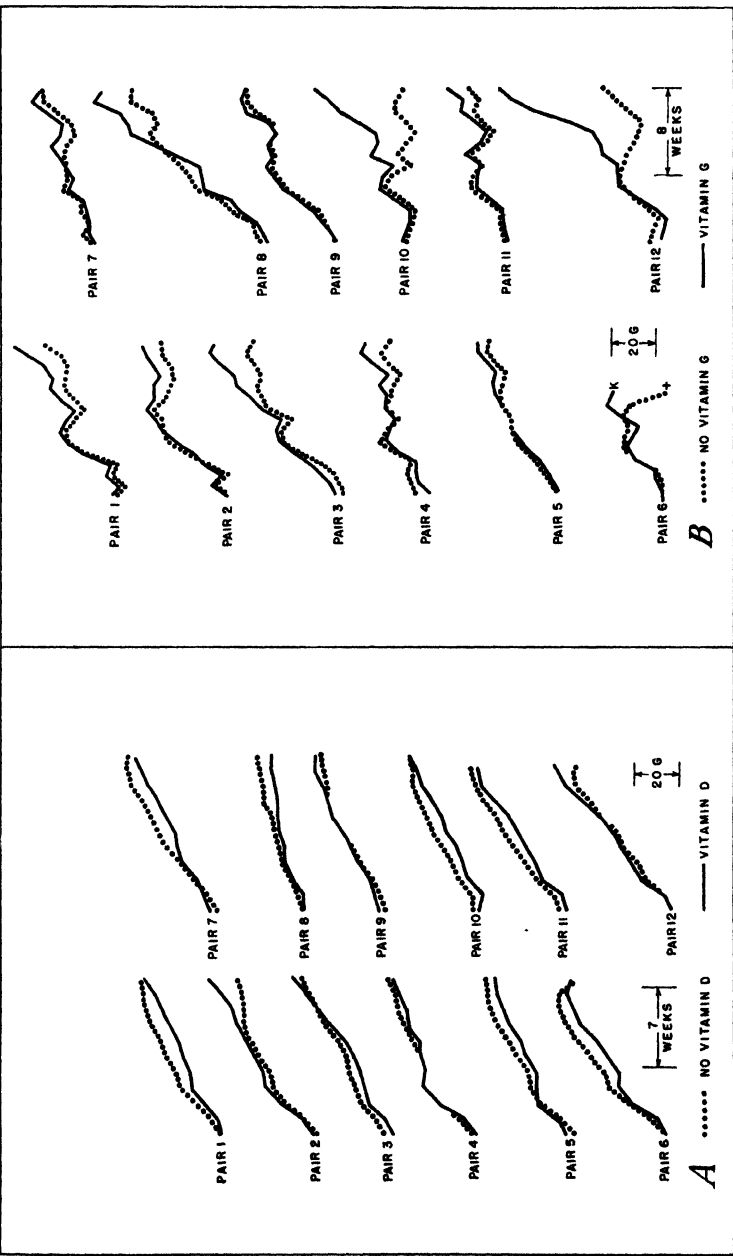


FIGURE 3.—Comparative growth of rats which received (A) Equicaloric quantities of a complete diet and of a diet deficient in vitamin D, and (B) diets rich and poor in vitamin B

the gain was protein (table 20), and there was also a greater heat loss (table 19) than there was with the rats on the complete diet.

There were no significant differences between the two groups as to the metabolizability of the energy of the rations (table 18), the digestibility of either the nitrogen or the energy of the rations (table 22), the gain in live weight (table 17), the nitrogen of the urine (table 21), the energy of the urine and feces, or the ratio of carbon to nitrogen in the urine (tables 21 and 22).

The carbon-nitrogen ratio of the urine was either 0.8 or 0.9 in every case, averaging 0.8+ with both groups of rats. The diet was high in protein (23.63 percent), and the ratio of carbon to nitrogen was about the same as in another experiment of this series in which the diet was about equally rich in protein.

THE EFFECTS OF VITAMIN G DEFICIENCY

The published work on vitamin G as affecting the utilization of food being meager and for the most part confined to observations on gain of live weight, an experiment was planned especially to determine the characteristic differences in the details of utilization of the protein and energy of food when supplied with and without adequate amounts of vitamin G. The method of experimentation was as in the foregoing studies of this series.

Kon (17) emphasized the necessity of determining the actual chemical composition and energy content of the gains in weight in investigations involving the relation of any indispensable nutrient to the metabolism of matter and energy; and in an earlier paper (16) suggested that vitamin G is intimately concerned with the general oxidative processes, though he later concluded (17) that its association with the metabolic processes of the body is merely comparable to that of other indispensable food ingredients.

St. Julian and Heller (27) found the digestibility of protein to be somewhat lower with rats which received a vitamin B₁ and a vitamin-B₂-deficient diet supplemented with vitamin B₂ alone, than when the diet was supplemented with vitamin B₁ alone, or with vitamin B₁ and B₂ together, or when no supplement was added. This finding, however, is not stressed by the authors, and they conclude that vitamins A, B₁, B₂, C, and D exert no effect on the digestibility of protein, fat, and carbohydrate.

Hassan and Drummond (13) noted that the heat-stable alkali-resistant fraction of the vitamin B complex in yeast extract renders very high protein diets adequate for growth.

Graham and Griffith (11) found a decreased utilization of food, for gain in live weight, when both vitamins B and G were supplied at a low level. They found that both vitamins were necessary for normal appetite, and that these two vitamins "seemed to depend upon the presence of each other for their activity", a finding which furnishes an example of the general interdependence of the values of food nutrients as stated by Forbes (6).

EXPERIMENTAL PROCEDURE

Twelve pairs of weanling rats, the pair mates being from the same litter and of the same sex, were fed, for 14 weeks, in a growth-, metabolism-, and body-analysis experiment.

The basal ration was composed as follows: Casein (vitamin-free) 18 percent, Osborne and Mendel salt mixture 4 percent, Crisco 10

percent, dextrin 64 percent, Cellu flour 2 percent, and cod-liver oil 2 percent. Vitamin B₁ was supplied in equal and sufficient amounts to both groups of rats in the form of a concentrate.⁵

Some difficulty was experienced in providing the desired difference in vitamin G content of the diet as fed to the two groups of rats. Autoclaved yeast was not suitable as a source of vitamin G because of accompanying protein and energy which it would contribute; and the use of yeast which had been exposed to ultraviolet rays seemed undesirable on account of the inconsistent results of this method of destruction of vitamin G, as reported in the literature. During the first 7 weeks of the experiment 2 methods of accomplishing the stated purpose were tried. A concentrate prepared from dried brewer's yeast having proved ineffective, an attempt was made to secure the desired difference in vitamin G by supplementing the diet for one group with untreated yeast, and for the other with a like amount of yeast previously heated, dry, at 100° C., to destroy vitamin G. Elevehjem and his associates (4), working with chicks, secured about 50 percent destruction of vitamin G by heating baker's yeast at 100° for 144 hours. In the light of this finding, samples of yeast were heated in a Freas oven at 100° for 14 and 29 days, respectively; but neither the growth (fig. 3, B) nor the appearance of the rats indicated vitamin G deficiency in the ration supplemented with the heated yeast. Block and Farquhar (2) have recently published a similar finding.

The supplement of dry heated yeast was, therefore, discontinued at the end of the seventh week, and an aqueous extract of Eli Lilly & Co.'s liver concentrate no. 343 was prepared according to the method of Guha (12). That liver contains an abundance of vitamin G has been quite well established, and there is evidence that it may also contain other factors especially favorable to growth and reproduction. It is recognized, therefore, that the water extract of the liver concentrate used in this experiment may have furnished more than a single factor in exerting its influence on the utilization of the protein and energy of the food.

Beginning with the eighth week (fig. 3, B) one rat of each pair received daily 0.5 cc of the extract, the high potency of which permitted its use in small amounts, thus minimizing the difference between the protein and energy intake of the two groups of rats. All supplements fed were carefully weighed and analyzed, and were considered in the computation of the intake of protein and energy. The rats given the supplemented diet received (on an average) 18 Calories (1.07 percent) more feed energy and 0.37 g (4 percent) more nitrogen than did their pair mates, in the course of the 14 weeks' experiment.

The rat of pair no. 6, which received the vitamin-G-deficient diet, died at the end of the tenth week, as a result of vitamin G avitaminosis, indicating that the dry-heating of the yeast used earlier in the experiment was not entirely ineffective in destroying vitamin G. The pair mate of this rat was killed for purposes of comparison.

In order to prevent more of the vitamin-G-deficient rats from dying, and to permit limited growth, small weighed amounts of yeast were added to the vitamin-G-deficient diet from time to time: observations on the condition and appearance of the rats were also made every few days and rectal temperatures of the rats were taken daily during the last 2 weeks of the experiment.

⁵ This concentrate was kindly donated by Parke, Davis & Co.

Various degrees of success have been reported by workers who have attempted to produce vitamin-G-deficiency dermatitis. At the end of this experiment there were 7 cases (out of a possible 12) of vitamin G avitaminosis, as indicated either by outward appearance or by autopsy, with individual variations as to symptoms and as to severity.

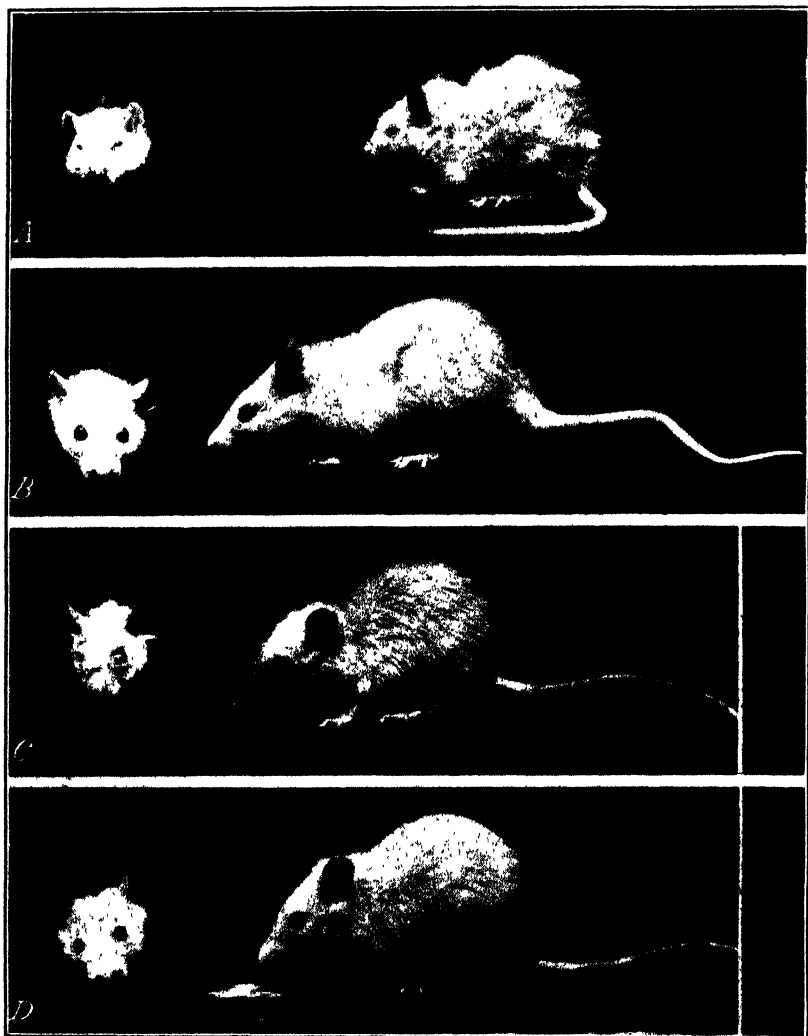


FIGURE 4 -Rats of pair nos 10 and 12, respectively, A and C being vitamin G deficient, and B and D being normal rats.

The deficient rats of pairs 3, 4, 7, and 11 developed mild pellagralike conditions, while the symptoms of the deficient rats of pairs 6, 10, and 12 resembled closely those reported by Goldberger and Lillie (9) and by Chick and Roscoe (3). The appearance of pairs 10 and 12 near the end of the experiment, is shown in figure 4.

Since coprophagy was encountered in a few cases, a wire tunnel was devised (fig. 5) which, when attached to the feed cup, prevented the rat from turning round during the taking of feed. While this device by no means wholly prevented the eating of the feces, it aided materially, as judged by the decrease in number of small particles of feces found in the crystallizing dish below the cage.

At the end of the experiment the rats were killed, and the contents of the alimentary tract were discarded as in the other experiments of this series. Observations made on the organs of the rats at this time revealed, in some cases, no difference between pair mates, but in pairs 3, 6, 7, 10, and 12 enteritis and accumulated intestinal gas were manifest, especially in the small intestine, these conditions being most marked in pairs 6, 10, and 12. All of the rats which received the liver supplement appeared healthy and normal throughout the experiment and at autopsy.

PRESENTATION OF RESULTS

A statistical study of the weekly gains in live weight revealed a superiority of performance by the rats which received the supplemented diet, the significance of which is indicated by odds of 1,000 to 1: also there were 516 refusals of feed, 356 of which were from the rats on the deficient diet. That the rats which received the vitamin G deficient diet limited the food consumption of their pair mates is, therefore, indicated by overwhelming odds. This fact, however, is not necessarily an indication that vitamin G has a direct, specific effect on appetite, but may signify only that the lesser food consumption is incidental to vitamin G avitaminosis.

Except for pair 6, the data in tables 23 to 28 represent an experimental period of 14 weeks. Early in the experiment there were no symptoms of vitamin G avitaminosis, probably because of a small amount of vitamin G in the deficient ration, and because of a body store of vitamin G. Well-defined results obtained from the whole period of 14 weeks, therefore, may be interpreted with confidence. Differ-

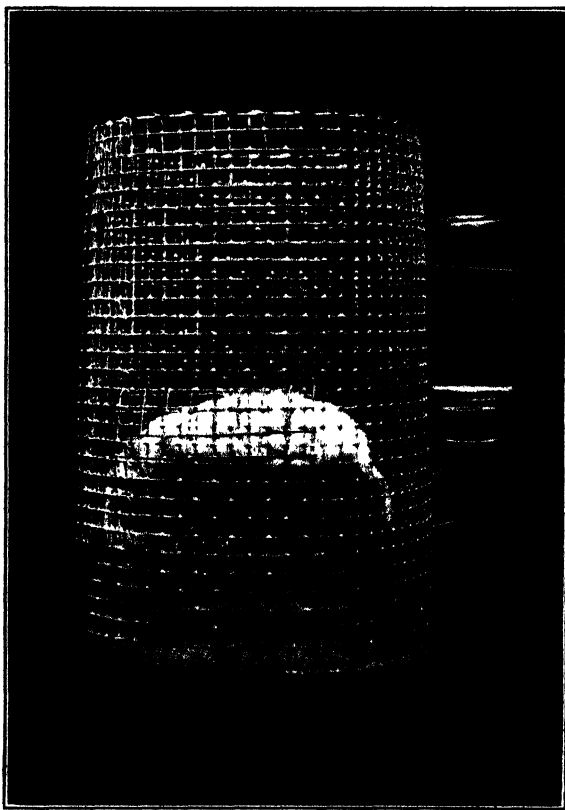


FIGURE 5 A rat eating inside a screen cylinder which diminished coprophagy.

ences in average values for the two groups of rats presented in the tables should be considered to have directional rather than quantitative significance, however, since the values obtained depend on the extent of the concurrent avitaminosis. In general, the greatest differences between pair mates shown in the tables correspond to the greatest differences between pair mates as judged by their behavior and general appearance—toward the end of the experiment and at autopsy.

TABLE 23.—Gain in body weight¹ of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to dry matter of food

Pair no.	Sex	Rats fed vitamin-G-supplemented diet			Rats fed vitamin-G-deficient diet		
		Food eaten (dry matter)	Gain in body weight	Ratio of gain to dry matter of food	Food eaten (dry matter)	Gain in body weight	Ratio of gain to dry matter of food
		Grams	Grams	Percent	Grams	Grams	Percent
1.....	♂	312.4	44.97	14.4	307.4	30.38	9.9
2.....	♀	346.8	38.07	11.0	342.0	28.84	8.4
3.....	♂	361.6	52.82	14.6	356.6	39.12	11.0
4.....	♀	318.8	28.76	9.0	313.9	14.18	4.5
5.....	♂	304.0	34.03	11.2	299.3	30.64	10.2
6.....	♀	230.0	24.85	10.8	226.5	5.00	2.2
7.....	♂	317.2	25.92	8.2	313.2	25.38	8.1
8.....	♀	452.6	75.61	16.7	447.9	59.05	13.2
9.....	♂	385.9	38.33	9.9	381.7	40.99	10.7
10.....	♀	333.4	38.92	11.7	329.9	1.61	.5
11.....	♂	338.8	26.57	7.8	335.0	16.76	5.0
12.....	♀	403.4	67.23	16.7	398.5	19.30	4.8
Average.....		342.1	41.34	11.8	337.7	25.94	7.1

¹ Contents of alimentary tract removed.

TABLE 24.—Energy of body gain of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to energy, and to metabolizable energy, of food

Pair no	Rats fed vitamin-G-supplemented diet					Rats fed vitamin-G-deficient diet					Body gain of energy of vitamin-G-deficient rats as percentage of pair mate
	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Food energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	
		Total	As percentage of food energy				Total	As percentage of food energy			
	Calories	Calories	Percent	Calories	Percent	Calories	Calories	Percent	Calories	Percent	Percent
1.....	1,553.3	91.2	5.9	1,432.5	6.4	1,532.1	45.4	3.0	1,415.4	3.2	49.8
2.....	1,724.8	106.0	6.1	1,581.1	6.7	1,705.4	74.2	4.4	1,555.0	4.8	70.0
3.....	1,798.8	96.4	5.4	1,656.1	5.8	1,778.5	59.9	3.4	1,641.1	3.6	62.1
4.....	1,584.8	70.6	4.5	1,447.2	4.9	1,564.9	31.1	2.0	1,430.9	2.2	44.1
5.....	1,510.8	61.3	4.1	1,387.6	4.4	1,491.8	65.1	4.4	1,341.6	4.9	106.2
6.....	1,143.4	38.7	3.4	1,056.0	3.7	1,129.2	-7.5	-	1,040.0	-	-
7.....	1,576.7	30.7	1.9	1,440.2	2.1	1,560.5	46.2	3.0	1,443.8	3.2	150.5
8.....	2,253.5	159.0	7.1	2,090.6	7.6	2,234.3	91.5	4.1	2,076.0	4.4	57.5
9.....	1,920.5	73.9	3.8	1,759.8	4.2	1,903.5	75.5	4.0	1,723.7	4.4	102.2
10.....	1,658.7	87.0	5.2	1,513.4	5.7	1,644.9	-6.5	-	1,436.9	-	-
11.....	1,684.7	59.8	3.5	1,549.4	3.9	1,660.3	31.2	1.9	1,615.0	2.1	52.2
12.....	2,007.7	153.1	7.6	1,858.9	8.2	1,987.0	33.0	1.7	1,819.1	1.8	21.6
Average.....	1,701.5	85.6	4.9	1,563.6	5.3	1,683.5	44.9	-	1,536.4	-	-

TABLE 25.—Heat loss of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to energy of food

Pair no	Rats fed vitamin-G-supplemented diet			Rats fed vitamin-G-deficient diet		
	Food energy	Heat loss		Food energy	Heat loss	
		Total	As percent-age of food energy		Total	As percent-age of food energy
	<i>Calories</i>	<i>Calories</i>	<i>Percent</i>	<i>Calories</i>	<i>Calories</i>	<i>Percent</i>
1.....	1,553.3	1,341.3	86.4	1,532.1	1,370.0	89.4
2.....	1,724.8	1,475.1	85.5	1,705.4	1,478.8	86.7
3.....	1,798.8	1,559.7	86.7	1,778.5	1,581.2	88.9
4.....	1,584.8	1,376.6	86.9	1,564.9	1,399.8	89.4
5.....	1,510.8	1,326.3	87.8	1,491.8	1,276.5	85.6
6.....	1,143.4	1,017.3	89.0	1,129.2	1,047.5	92.8
7.....	1,576.7	1,409.5	89.4	1,560.5	1,397.6	89.6
8.....	2,253.5	1,921.6	85.3	2,234.3	1,984.5	88.8
9.....	1,920.5	1,685.9	87.8	1,903.5	1,648.2	86.6
10.....	1,658.7	1,426.4	86.0	1,644.9	1,443.4	87.8
11.....	1,684.7	1,489.6	88.4	1,669.3	1,483.8	88.9
12.....	2,007.7	1,705.8	85.0	1,987.0	1,786.1	89.9
Average.....	1,701.5	1,477.9	87.0	1,683.5	1,491.5	88.7

TABLE 26.—Nitrogen of body gain of rats fed vitamin-G-supplemented or vitamin-G-deficient diets as related to fat and energy of body gain and to nitrogen of food

RATS FED VITAMIN-G-SUPPLEMENTED DIET

Pair no	Nitrogen of body gain	Fat gained		Energy gained			Nitrogen of food	
		Total	Per gram of nitrogen gained	Total	As fat	As protein	Total	Utilized for body gain
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Grams</i>	<i>Percent</i>
1.....	1.87	3.0	1.6	91.2	27.0	64.2	8.81	21.2
2.....	1.60	5.8	3.6	106.0	53.2	52.8	9.74	16.4
3.....	2.23	2.7	1.2	96.4	24.7	71.7	10.14	22.0
4.....	1.40	2.8	2.0	70.6	25.7	44.9	8.96	15.6
5.....	1.63	1.0	.6	61.3	9.1	52.2	8.60	19.0
6.....	1.23	0.0	0.0	38.7	0.0	38.7	6.47	19.0
7.....	1.18	—	—	30.7	—7.3	38.0	8.93	13.2
8.....	2.80	7.2	2.6	159.0	66.7	92.3	12.51	22.4
9.....	1.56	2.4	1.5	73.9	23.0	50.9	10.74	14.5
10.....	1.54	3.9	2.5	87.0	36.6	50.4	9.37	16.4
11.....	1.21	2.2	1.8	59.8	21.1	38.7	9.51	12.7
12.....	2.68	7.0	2.6	153.1	65.8	87.3	11.23	23.9
Average.....	1.74	3.1	—	85.6	28.8	56.8	9.58	18.0

RATS FED VITAMIN-G-DEFICIENT DIET

1.....	1.48	—0.2	—	45.4	—2.4	47.8	8.43	17.6
2.....	1.27	3.5	2.8	74.2	31.4	42.8	9.32	13.6
3.....	1.76	.4	.2	59.9	3.0	56.9	9.70	18.1
4.....	.98	.3	.3	31.1	2.1	29.0	8.57	11.4
5.....	1.34	2.2	1.6	65.1	19.5	45.6	8.19	16.4
6.....	.49	—2.3	—	—7.5	—20.5	13.0	6.17	7.9
7.....	1.14	.9	.8	46.2	8.4	37.8	8.58	13.3
8.....	2.38	1.4	.6	91.5	13.2	78.3	12.10	19.7
9.....	1.61	2.5	1.6	75.5	23.8	51.7	10.37	15.5
10.....	.30	—1.7	—	—6.5	—15.7	9.2	9.07	3.3
11.....	.82	.1	.1	31.2	1.3	29.9	9.19	10.0
12.....	1.01	0.0	0.0	33.0	1.0	32.0	10.88	9.3
Average.....	1.22	0.6	—	44.9	5.4	39.5	9.21	13.0

TABLE 27.—*Urinary carbon of rats fed vitamin-G-supplemented and vitamin-G-deficient diets as related to urinary nitrogen*

Pair no	Rats fed vitamin-G-supplemented diet			Rats fed vitamin-G-deficient diet		
	Urinary carbon	Urinary nitrogen	Carbon-nitrogen ratio	Urinary carbon	Urinary nitrogen	Carbon-nitrogen ratio
	Grams	Grams		Grams	Grams	
1	4.27	6.29	0.68	5.00	6.28	0.80
2	4.66	7.58	.61	5.09	7.03	.72
3	3.93	7.01	.56	4.89	6.89	.71
4	4.74	6.98	.68	5.56	6.92	.80
5	4.05	6.45	.63	4.62	6.36	.73
6	2.41	4.79	.50	2.92	5.07	.58
7	4.10	6.65	.63	4.61	6.37	.72
8	5.19	8.65	.60	5.33	8.72	.61
9	5.16	8.17	.63	4.82	7.09	.63
10	4.39	6.99	.63	10.93	6.92	1.59
11	4.51	7.41	.61	5.76	7.35	.78
12	4.86	7.68	.63	5.92	8.48	.70
Average	4.38	7.06	.62	5.46	7.01	.74

TABLE 28.—*Digestibility of nitrogen and energy-producing nutriment of diets containing or lacking vitamin G, when fed to rats*

RATS FED VITAMIN-G-SUPPLEMENTED DIET

Pair no	Nitrogen			Energy		
	Food	Feces	Digested	Food	Feces	Digested
	Grams	Grams	Percent	Calories	Calories	Percent
1	8.81	0.56	93.6	1,553.3	71.8	95.4
2	9.74	.62	93.6	1,724.8	89.8	94.8
3	10.14	.76	92.5	1,798.8	95.4	94.7
4	8.96	.67	92.5	1,584.8	83.5	95.0
5	8.60	.59	93.1	1,510.8	75.5	95.0
6	6.47	.55	91.5	1,143.4	57.3	94.6
7	8.93	.70	92.2	1,576.7	85.0	95.1
8	12.51	.88	93.0	2,253.5	111.5	94.8
9	10.74	.69	93.6	1,020.5	100.1	94.3
10	9.37	.81	91.4	1,658.7	94.0	95.1
11	9.51	.75	92.1	1,681.7	82.1	95.4
12	11.23	.72	93.6	2,007.7	91.8	94.9
Average	9.58	.69	92.7	1,701.5	86.5	94.9

RATS FED VITAMIN-G DEFICIENT DIET

1	8.43	0.46	91.5	1,532.1	59.8	93.1
2	9.32	.61	93.1	1,705.4	94.5	94.5
3	9.70	.56	94.2	1,778.5	81.4	95.4
4	8.57	.57	93.3	1,564.9	71.9	95.5
5	8.19	.63	92.3	1,491.8	97.5	93.5
6	6.17	.50	91.9	1,129.2	53.6	95.0
7	8.58	.53	93.8	1,560.5	64.0	95.9
8	12.10	.71	94.1	2,234.3	95.4	95.7
9	10.37	.84	91.9	1,903.5	122.6	93.6
10	9.07	.88	90.3	1,644.9	94.8	94.2
11	9.19	.79	91.4	1,660.3	89.0	94.7
12	10.88	.90	91.7	1,987.0	98.6	95.0
Average	9.21	.67	92.7	1,683.5	85.3	94.9

The rats which received the supplemented diet averaged 0.29° C. higher in body temperature than did their pair mates, but statistical treatment of the data reveals odds of only 4 to 1 that this difference did not occur by chance. It may be pointed out, however, that this

finding does not contra-indicate a lower level of general oxidative metabolism, inasmuch as an abnormal body temperature signifies a disturbance of the heat-regulatory mechanism rather than a change in heat production. Findlay (5) has reported a drop in body temperature as a terminal feature of vitamin G avitaminosis.

The superiority of the gains in body weight of the rats which received the vitamin-G-supplemented diet is shown in table 23, the odds being about 525 to 1 that a given amount of the complete diet produced more gain in body weight than did a like amount of a similar diet which was deficient in vitamin G. No marked differences in live weight developed until the supplement of liver extract was added (fig. 1, *D*).

The body gain in energy (table 24) was, on an average, significantly greater in the case of the rats which received the vitamin-G-supplemented diet, though in three cases (pairs 5, 7, and 9) the difference appeared to be in favor of the deficient diet. The deficient rats of these three pairs, throughout the experiment, exhibited little evidence of avitaminosis. Conversely, the bodies of two of the rats (from pairs 6 and 10) on the deficient diet contained less energy at the end of the experiment than at the beginning; and these two pairs exhibited the greatest differences in appearance between pair mates, both before death and at autopsy.

The deficient rat of pair 6, which died at the end of the tenth week, showed lesions on the paws and nose, a biometrical symmetry in loss of hair, a scaliness of the skin where the hair had fallen out, and dermatitis over the entire body. At autopsy there were noted enteritis, especially of the ileum, gas in stomach and intestine, and mucuous colitis in the small intestine. The mate of this rat, killed when the deficient rat died, appeared to be normal.

The appearance of the rats of pairs 10 and 12, at about the end of the experiment, is shown in figure 4. It is to be noted (table 24) that the rats of these pairs show marked differences in the body gain of energy. The significance of the greater storage of energy by the rats which received the vitamin-G-supplemented diet, on the basis of the data in table 24, is indicated by odds of about 400 to 1.

The heat loss (table 25) was somewhat higher with the deficient rats than with the pair mates, the significance of the difference being expressed by odds of 7 to 1.

The division of the gain of energy into fat and protein (table 26) is based on the actual energy as determined in the ether extract and on the nitrogen of the extraction residue of the rat bodies at the end of the experiment, both of these values being corrected for the amount of fat and protein in the rats at the start of the experiment, as determined by the analysis of a control group of 10 similar rats.

It is of interest to note that the calories of energy gained, as measured in the body extraction residue, compare favorably with a computed value obtained by multiplying the grams of protein ($N \times 6.00$) gained by the factor 5.7. In the case of the rat which died during the experiment there is a considerable discrepancy, but with the remaining 23 rats the computed value compares well enough with the observed value (average, 105.7 percent) to justify the designation of fat and protein to the ether extract and the extraction residue, respectively.

On an average, 66.4 percent of the total energy stored by the vitamin-G-supplemented rats was in the form of protein, whereas

88.0 percent of the total stored by their pair mates was in this form. Thus, though the total gain of energy and the gain of energy as protein are considerably greater with the rats which received the supplemented diet, their pair mates were relatively more restricted in their ability to store fat than they were to store protein. Whether this fact is a characteristic effect of vitamin G deficiency, or whether it merely represents the strong inherent tendency of the young animal to synthesize protein, is not indicated.

There was no significant difference in the digestibility of the protein and energy of the two rations (table 28).

The carbon-nitrogen ratios in the urine are given in table 27. The significance of the higher ratios obtained from the deficient rats is indicated by odds of 35 to 1. These results agree with those of Kon (16), who obtained an increased carbon-nitrogen ratio in the urine of rats kept on a diet deficient in vitamin G. The higher carbon-nitrogen ratios result from a higher amount of carbon in the urine, the nitrogen content of urine from the two groups being almost identical. Lack of vitamin G seems to prevent the normal oxidation of carbon compounds, which then appear, in increased quantities, in the urine.

The energy of the urine of the rats which received the supplemented diet averaged 51.4 calories, as compared with 61.8 calories for their pair mates, the significance of the difference being expressed by odds of 32 to 1.

SUMMARY

Two metabolism- and body-analysis experiments, of about 90 and 107 days' duration, respectively, were conducted with 32 rats, fed by the paired method, for the comparison of white and yellow corn, as components of approximately complete diets, as affecting the utilization of nitrogen- and energy-producing nutrients.

The rats which received white corn in the first experiment manifested xerophthalmia after 90 days' feeding; but the rats which received white corn in the second experiment did not show this disorder.

In the first experiment the yellow-corn ration was superior to the white in respect to (1) palatability, (2) gain in weight, (3) gain in energy, and (4) proportion of the energy gained which was present as fat. On the other hand, the white-corn ration was superior to the yellow in (1) digestibility of food nitrogen and (2) digestibility of food energy.

In the second experiment the yellow-corn diet was shown to be definitely superior to the white corn in (1) palatability, (2) gain in live weight, (3) grams of nitrogen gained, (4) percentage of the energy gained as protein, and (5) percentage of food nitrogen stored. The greater gain in live weight of the rats fed yellow corn consisted of protein and water. The rats fed white corn exceeded the rats fed yellow corn, however, in (1) digestibility of the food energy, (2) percentage of food energy gained as fat, (3) fat gained per gram of nitrogen gained, (4) elimination of carbon in the urine, and (5) elimination of nitrogen in the urine.

Considering the two experiments together, the yellow-corn ration definitely excelled the white-corn ration only in palatability and in gain produced in body weight; while the white-corn ration excelled in digestibility of food energy. In other respects the two experiments,

so far as they covered the same observations, were not in definitely significant agreement.

While yellow corn is normally more nutritious than white corn and normally contains much more vitamin A, and while the particular lots of yellow and of white-corn used in these experiments differed as to nutritive value, it is considered obvious, in the light of these results and of common knowledge of the composition of corn, that the difference in color of white and yellow corn is not positively indicative of the usual differences in nutritive value.

The effects of vitamin A deficiency on the utilization of energy-producing nutriment and protein were studied in a growth-, metabolism-, and body-analysis experiment with 24 weanling albino rats, which were fed, by the paired feeding method, for 84 days, in a comparison of a complete diet with a similar one which differed only in being deficient in vitamin A—the caloric intake being the same with both groups.

The vitamin-A-deficient rats were maintained in only moderate A depletion, and the experiment was terminated soon after avitaminosis had become acute and general among the deficient group.

The complete diet exceeded the vitamin-A-deficient diet only in palatability and in gain in live weight and in nitrogen.

The rats on the vitamin-A-deficient diet eliminated slightly more carbon and energy in the urine than did the rats on the complete diet, but there were no statistically significant differences between the performance of the two groups with respect to digestibility or metabolizability of the diets, nitrogen in the urine, heat production, gain of fat or of energy, or gain of fat per gram of nitrogen gained.

In its early stages the most prominent effect of vitamin A deficiency was to depress the appetite.

The effects of vitamin D deficiency on the utilization of energy-producing nutriment and protein were studied, with the albino rat, by the paired feeding method, in a growth-, metabolism-, and body-analysis experiment of 10 weeks' duration.

The differences in the response of the rats to the complete and the vitamin-D-deficient diets were compared by statistical analysis.

With equicaloric intake by both groups, the rats on the vitamin-D-deficient diet exceeded their pair mates on the complete diet in utilization of the food nitrogen for body increase, in the percentage of the body gain which was protein, and in heat loss.

The rats on the complete diet exceeded their pair mates on the vitamin-D-deficient diet in appetite, gain of fat and energy, and elimination of carbon in the urine.

There were no significant differences between the two groups of rats in the metabolizability of the energy of the diets, the digestibility of either the nitrogen or the energy of the diets, the gain in live weight, the nitrogen of the urine, the energy of the urine and the feces, or the ratio of carbon to nitrogen in the urine.

The diets contained 23.63 percent protein; and the ratio of carbon to nitrogen, in the urine, with the rats on the vitamin-D-deficient as well as those on the complete diet, was 0.8+.

Twelve pairs of young growing rats were fed by the paired feeding method for 14 weeks, with quantitative collection of feces and urine, and analysis of the rat bodies, to determine the effects of vitamin G deficiency on the utilization of the food protein and energy.

A water extract of a liver concentrate proved to be a satisfactory source of vitamin G, and a small amount of this extract was given daily to one rat of each pair.

The deficiency of vitamin G depressed the appetite, the growth, the synthesis of protein and of fat, and the storage of energy.

The deficiency of vitamin G did not appreciably affect the digestibility of the protein and energy of the diet, nor did it exert any certain effect on the heat production.

The deficiency of vitamin G had the effect of increasing the carbon-nitrogen ratio in the urine, thus indicating a relationship between the oxidative processes and this vitamin.

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ERGOT AS A FACTOR IN THE NUTRITIVE VALUE OF RYE FOR RATS AND SWINE¹

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INTRODUCTION

In view of the numerous reports (4, 6, 7, 8, 9)² of cases of ergotism both in livestock and human beings, and especially in view of the fact that rye is but rarely free from ergot (*Claviceps purpurea* (Fr.) Tul.), it is surprising that its effect upon the nutritive value of rye has never been thoroughly investigated. So far as the writers are aware, the only investigation of this nature which has any significance from the standpoint of livestock feeding is that of Sackville and Sinclair.³ These authors reported that rye containing 1.46 percent of ergot was worth only 92 percent as much as pure rye, for the former was so distasteful that it was impossible to get the pigs to eat sufficient quantities of it to make satisfactory gains.

In a previous investigation (3), it was found that when the ergot was removed from rye the rye was eaten readily, even when not mixed with protein supplements. Furthermore, there was never any indication of the disturbances which so commonly occur when rye is fed to swine. The possibility that such disturbances might be due to the small amounts of ergot which are usually present in rye was investigated with both rats and swine. The results of the investigation are presented in this paper. Since a review of the literature has already been given (3), it is omitted here.

EXPERIMENTS WITH RATS

EFFECT OF 1 PERCENT OF ERGOT UPON GROWTH

It was desired to determine first whether ergot has any effect upon growth when the food intake of ergotized diets and of basal diets are equalized for pairs of rats of the same age, weight, and sex. Accordingly, ergot obtained from a drug company was incorporated at a 1 percent level in a diet containing rye and at the same level in a purified diet. The 1 percent of ergot replaced 1 percent of dextrin in each basal diet. The basal diet in which rye was used consisted of 91.74 percent of ergot-free rye, 4.76 percent lard, 2.50 percent salt mixture, and 1 percent dextrin. The purified diet consisted of 66 percent dextrin, 20 percent casein, 10 percent lard, and 4 percent salt mixture. Six pairs of male rats were fed the rye diets and 3 pairs of females and 1 pair of males were fed the purified diets. Each rat used in these experiments, as well as in the experiments that followed, was given daily in addition to the basal diet a supplement of 500 mg of yeast tablets

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² Reference is made by number (italic) to Literature Cited, p. 45.

³ SACKVILLE, J. P., and SINCLAIR, R. D. BARLEY VS. RYE FOR FATTENING PIGS. Alberta Univ. Dept. Anim. Husbandry Mimeographed Rept. Expt. 49. 1923.

which had been immersed in cod-liver oil. In all diets the salt mixture used was that of Hawk and Oser (2).

The composite growth curves of the rats of the first two experiments are shown in figure 1, *A* to *D*. The lack of growth of the rats receiving the rye diets and the slight growth of those receiving the purified diets was occasioned by low food intake. The former consumed 17.5 g and the latter 25.06 g of food per rat per week during 4 weeks.

Although the addition of ergot apparently rendered the rye diet more unpalatable than the purified diet, a similar relationship has

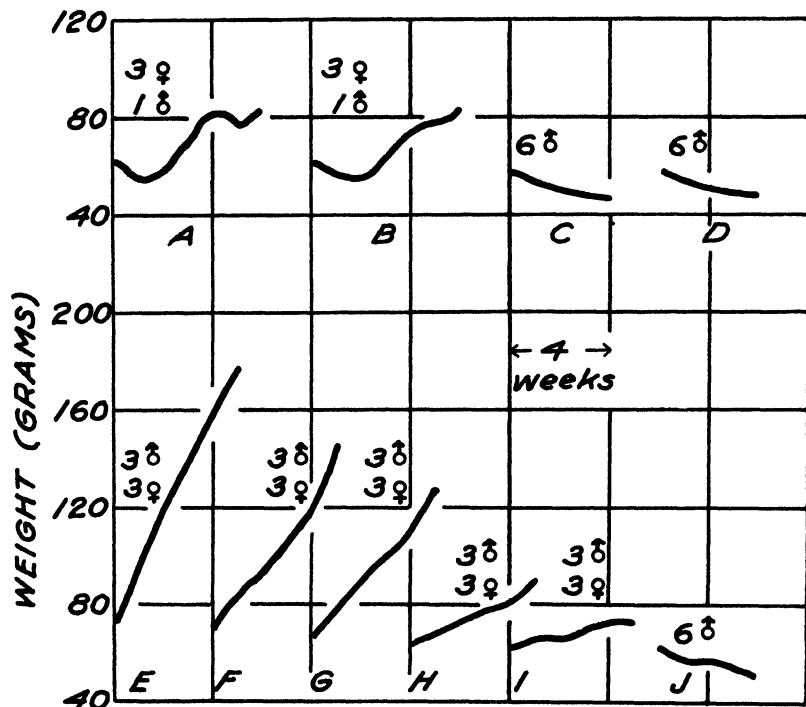


FIGURE 1—Average growth of groups of rats fed different rations. *A*, Purified basal ration, *B*, purified basal ration plus 1 percent ergot; *C*, rye basal ration, *D*, rye basal ration plus 1 percent ergot; *E*, rye basal ration plus liver meal, *F*, rye basal ration, *G*, rye basal ration plus liver meal plus 0.1 percent ergot, *H*, rye basal ration plus liver meal plus 0.5 percent ergot, *I*, rye basal ration plus liver meal plus 1 percent ergot, *J*, rye basal ration plus 1 percent ergot

been found between the consumption of a diet containing 90 percent or more of ergot-free rye and the consumption of a purified diet when both were offered ad libitum. This difference in food consumption may not have been entirely a matter of palatability but may have been due in part to the higher protein content of the purified diet.

Four of the rats that received the rye diet containing ergot and two of the controls had died of starvation by the end of 6 weeks. There was no mortality among the rats that received the ergot as a part of the purified diet. Growth of the controls in each experiment was but little better than that of their pair mates because of their restricted food allowance. There were no outward manifestations of ergotism in any of these rats.

**EFFECT OF LIVER MEAL IN OVERCOMING UNPALATABILITY OF DIETS CONTAINING
ERGOT AT 1-, 0.5-, AND 0.1-PERCENT LEVELS**

Because of the pronounced effect of 1 percent of ergot upon food intake an effort was made to determine the effect upon food consumption of lower percentages of ergot and to find whether a liver meal (a special product made from livers) which had previously been shown to enhance greatly the palatability of the purified diet and of diets containing rye (3), would overcome the distastefulness of ergot-treated rations.

The basal diet consisted of the following: Rye, 90 percent; dextrin, 7.32 percent; and salt mixture, 2.68 percent. Five modifications of this diet were fed: (1) with 1 percent of ergot; (2) with 6.32 percent of liver meal; and (3-5) with 6.32 percent of liver meal and 1.0, 0.5, and 0.1 percent, respectively, of ergot. The ergot or liver meal and ergot replaced an equivalent weight of dextrin in each diet. After the second week the liver meal in the diet containing 6.32 percent liver meal and 1 percent ergot was increased to 20 percent (fig. 1, *G*). Six rats, 3 males and 3 females, were fed each diet, except the first modification, which was fed to 6 males. All diets were consumed ad libitum.

The composite growth curves for the rats in this series of experiments are given in figure 1, *E* to *J*. An inspection of these curves reveals that the liver meal, even when comprising 20 percent of the diet, did not overcome the distaste of the rats for the diets containing ergot and that ergot retarded growth even when fed as 0.1 percent of the diet. The addition of the liver meal to the rye basal diet caused a pronounced increase in the food consumption and growth of the rats, as shown in table 1.

TABLE 1 - *Average weekly quantity (grams) of food consumed by rats in a comparison of the effect of ergot when fed with rye*

Ration and sex of rats in groups	Food consumed for week indicated				
	1	2	3	4	5
Basal (3 male, 3 female)	58.6	58.6	68.3	72.1	75.8
Basal + 1.0 percent ergot (6 male)	26.1	21.8	22.2	19.6	
Basal + liver meal (3 male, 3 female)	80.1	107.3	96.6	91.3	100.5
Basal + liver meal + 1.0 percent ergot (3 male, 3 female)	31.1	28.6	52.0	25.4	18.7
Basal + liver meal + 0.5 percent ergot (3 male, 3 female)	46.8	37.2	51.8	41.2	54.6
Basal + liver meal + 0.1 percent ergot (3 male, 3 female)	65.8	75.8	71.8	61.6	79.8

GROWTH OF RATS RECEIVING CORN AND RYE DIETS WITH AND WITHOUT ERGOT

An effort was made to find whether a diet made up of ergot and corn would be as unpalatable as a diet of ergot and rye.

The rye diet contained 82.17 percent rye; 12.47 percent "trinity" (2 parts tankage, 1 part linseed meal, and 1 part alfalfa meal); 1.97 percent salt mixture; 0.53 percent agar; 1.96 percent lard; and 1.0 percent dextrin. The corn diet contained 79.27 percent corn; 12.47 percent trinity; 1.97 percent salt mixture; 0.98 percent agar; 1.08 percent lard; and 4.23 percent dextrin. These diets were isodynamic. They were fed ad libitum and also in a paired feeding test. Two other groups of rats were fed each of the basal diets in which 0.5 and 1 percent, respectively, of ergot replaced an equivalent weight of dextrin. This ergot was not from the same supply as the ergot used in the experiments reported in sections 1 and 2 but was purchased from the

same company. Three male and three female rats were fed each diet. The growth and food consumption data for this series of experiments are tabulated in table 2.

TABLE 2.—Average weekly quantity (grams) of food consumed and gain in weight by rats for 4-, 8-, 10-, and 12-week periods in the comparison of the effect of ergot in diets of corn and rye

Ration	4 weeks		8 weeks		10 weeks		12 weeks	
	Food	Gain	Food	Gain	Food	Gain	Food	Gain
Corn diet+1.0 percent ergot	50.5	11 0	59 7	13 6	61.9	12.6	63 4	11.5
Rye diet+1 0 percent ergot	54.0	12 7	58 0	12.0	60 4	11 4	61 0	9.9
Corn diet +0.5 percent ergot	60 4	15 0	70 7	16.0	73.1	14.5	75 0	13 3
Rye diet+0.5 percent ergot	52.6	13.5	61.3	12.7	63.9	11.4	66.4	10 6
Corn basal diet (ad libitum)	72.7	19.7	82.6	18.5	84.4	16 6	85 5	15 0
Rye basal diet (ad libitum)	73 0	18.5	81 8	17.0	83.0	14.9	85 9	14.4
Corn basal diet (paired)	63.6	14 3	74 0	14 8	75 1	13.4		
Rye basal diet (paired)	63.6	15.2	74 0	14.5	75 1	13.3		

The fact that all the rats on the rye diets containing 1.0 percent of ergot grew fairly well was surprising in view of the negligible growth and high mortality of the rats on the rye plus liver diets containing 1.0 percent of the ergot used in the first ergot experiments. There were no outward manifestations in any of the animals which could be considered as due to the ergot. There must indeed be great differences between different samples of ergot in the amount of the principles present which account for its distastefulness. Apparently this has been recognized by livestock feeders, for McNeil and Pammel (5), referring to ergotized hay, state: "Such hay is not always dangerous even when infested with ergot * * * ." There is but little indication that the ergot was more detrimental when fed with rye than when fed with corn. That there was nothing in the rye used in this experiment which retarded food consumption or growth is shown by the results of feeding the basal diets of corn and rye both ad libitum and at the same level of food intake.

EXPERIMENTS WITH SWINE

The only rye diets fed to rats which gave poor growth were those containing ergot. These experiments indicated that ergot might be responsible for the unsatisfactory results when rye is fed to swine. Accordingly, two experiments were carried out to obtain light upon this phase of the question.

EFFECT OF 1 PERCENT OF ERGOT UPON GROWTH

In the first experiment the effect of ergot upon growth was investigated. Six pairs of pigs were used. One pig of each pair received a rye ration while its pair mate received the same amount of the same ration to which had been added 1 percent of ergot. The rye used contained only a trace of ergot. The pigs were fed twice daily in individual crates.

The basal ration consisted of rye 91.16 percent, liver meal 7.36 percent, mineral mixture 1.48 percent, plus 10 cc of cod-liver oil daily per pig.

The ergot was hand-picked from some elevator screenings. It appeared to be a mixture of ergot from the various grains. Before feeding it in this experiment it was fed to several rats as a part of a

rye diet and found to reduce greatly their food intake, as in the first of the rat experiments. The mineral mixture employed was that fed to the station swine herd, and consisted of limestone 50.00 percent, bone meal 27.97 percent, 20.00 percent salt, iron oxide 2.00 percent, copper sulphate 0.01 percent, and potassium iodide 0.02 percent.

The ration containing the ergot was very distasteful to the pigs for several weeks after the experiment began. One pig ate such small quantities that it barely maintained its weight for 4 weeks, and another ate so little that it just maintained its weight for 6 weeks. Most of the pigs seemed gradually to accustom themselves to the ergot, although there was considerable variation in this respect, as is evidenced by the growth and feed-consumption data in table 3.

TABLE 3.—*The effect of 1 percent of ergot in a rye ration upon the growth of pairs of swine over a period of 12 weeks*

[Weights in pounds]				
Pair and ration	Initial weight	Final weight	Total gain	Total feed
Pair 1, males				
Control	70 3	143.3	73.0	223
Ergot	68 6	132.6	64 0	223
Pair 2, males				
Control	56 3	113 6	57 3	151 7
Ergot	53 6	89 3	35 7	151.7
Pair 3, males				
Control	47 6	92.3	44 7	106 5
Ergot	46 0	68.0	22.0	106.5
Pair 4, females				
Control	51 6	112.3	60 7	162 5
Ergot	51 0	93.3	42 3	162 5
Pair 5, females				
Control	46 6	103 6	57 0	175 5
Ergot	48 3	95.0	46.7	175.5
Pair 6, females				
Control	64 3	145 6	81.3	241.7
Ergot	66 0	124 0	58 0	241 7

A statistical study was made of the data in table 3 according to the method of student (1). The results were as follows:

Mean difference in total gain in weight between pair mates	pounds	17.55
Standard deviation		5.807
Z value		3.0
Probability		0.9994

That the mean difference of 17.55 pounds per pig between pair mates was due to the ergot in the ration of the experimental pig is shown by the statistical study. The probability of 0.9994 gives odds of over 1,000 to 1 that this was the case. While this experiment shows conclusively that ergot retards growth over and above any effect upon feed consumption, it does not, of course, tell to what extent feed consumption was retarded by this percentage of ergot. Throughout the experiment both pigs of each pair showed signs of hunger, and that they were hungry is indicated by the fact that the daily feed consumption was only 2.1 pounds per pig. Pigs weighing as much as those used in this experiment should eat at least twice that amount.

EFFECT OF ONE-HALF OF 1 PERCENT OF ERGOT UPON FEED CONSUMPTION

In order to determine whether aging causes ergot to lose its property of imparting distastefulness to a ration and also to determine the effect of a lower level of ergot upon feed consumption, another experiment was performed 1 year later in which the ergot was from the same supply that had been used in the first experiment.

Three pairs of pigs of an average weight of 50.6 pounds were selected for this experiment. Although they were paired as to sex, litter, and weight, both pigs of each pair were allowed to eat all the feed for which they cared twice each day. One pig received the basal ration consisting of rye 91.16 percent, liver meal 7.36 percent, and the mineral supplement 1.48 percent. Its pair mate received the basal ration plus 0.5 percent of ergot. Both pigs also received 10 cc of cod-liver oil daily. At the end of 3 weeks the rations were reversed for the same length of time. The rye used was entirely free of ergot.

TABLE 4.—*The effect of 0.5 percent of ergot in a rye ration upon the food consumption (pounds) per pig per week, of pairs of swine, over a period of 6 weeks*

Pair and ration	Food consumption					
	First week	Second week	Third week	Fourth week ¹	Fifth week	Sixth week
Pair 1, female						
Basal	15.7	18.3	21.5	19.1	21.8	28.0
Ergot	7.1	14.2	15.4	8.9	15.1	16.5
Pair 2, male:						
Basal	10.0	14.3	15.7	15.9	27.1	26.7
Ergot	6.0	11.6	15.2	8.4	15.2	16.7
Pair 3, female						
Basal	11.8	16.9	18.1	9.4	19.1	18.5
Ergot	7.3	10.0	10.8	7.6	9.7	8.0

¹ Rations were reversed at end of third week.

As shown by the feed-consumption data in table 4, the 0.5 percent of ergot had a marked effect upon the palatability of the ration. For the 6 weeks only 62.1 percent as much of the ergot ration was eaten as of the basal ration. Again, as in the first experiment a year earlier, there was a noticeable difference in the apparent distastefulness of the ergot to the various pigs. Also, as in the first experiment, there was a gradual tendency for the pigs to overcome their dislike for the ration containing the ergot. The average daily amount of the basal ration eaten for the 6-week period was 2.64 pounds. This daily feed consumption, which is rather low, is, however, somewhat higher than that eaten by a similar group of pigs when the trinity mixture was used as the protein supplement instead of liver meal and the feeds were self-fed instead of fed twice daily as was done in this experiment. Liver meal was fed with the rye in both ergot experiments in order to insure as satisfactory an intake of rye as possible and to determine whether or not a palatable protein supplement would overcome the distastefulness of the ergot. The results of feeding the liver meal in the swine experiments were in entire agreement with those obtained in the rat experiments.

SUMMARY AND CONCLUSIONS

The effects of ergot upon the growth and food consumption of rats and swine were investigated. To determine the effect of ergot upon the growth of rats it was fed in two paired-feeding experiments as 1.0 percent of a purified diet and at the same percentage of a diet containing 91.74 percent of rye. In the swine experiment one paired-feeding test was carried out in which the controls received a rye basal diet and the experimental pigs received the same diet in which 1 percent of the rye had been replaced by ergot. For the rats the rye diet

containing the ergot was so unpalatable that some of the rats in these paired-feeding tests, controls as well as experimentals, had died of starvation by the end of 6 weeks. Although the purified diet containing the ergot was less unpalatable than the ergotized rye diet the food intake in both experiments was at such a low level that there was little difference in growth between the rats of a pair. In the swine experiment the ergot retarded growth to a statistically significant degree.

The effect of ergot upon the food consumption of rats was studied by feeding it as 1 percent of a diet containing rye and as 1, 0.5, and 0.1 percent of diets containing rye and liver meal. Ergot from another supply was fed as 1 percent and 0.5 percent of a diet containing rye and of a diet containing corn as the cereal. One year after the first swine experiment ergot from the same supply was fed to swine as 0.5 percent of a rye ration. In these experiments with rats the first ergot that was fed retarded food consumption at all three levels, the effect diminishing as the level of ergot was decreased. The liver meal which greatly increased the consumption of the rye basal diet did not overcome the apparent distastefulness of the diets containing the ergot. In the second ergot experiment with rats the growth and food consumption, although somewhat subnormal, were surprisingly high. Ergot appears to vary considerably in the content of the principles which cause its distastefulness. There was but little difference in the food consumption and growth of the rats receiving the rye and ergot diets as compared with those receiving the corn and ergot diets. In the swine experiment the ergot had not lost its distastefulness after 1 year in storage, for at a 0.5-percent level, feed consumption was retarded.

There were no gross symptoms of ergotism in any of the experimental animals. There would seem to be little danger of this disease occurring in swine that are fed grain containing ergot because of their pronounced distaste for feeds infected with this fungus.

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FIELD REACTION OF VARIETIES AND SELFED LINES OF CORN TO DIFFERENT COLLECTIONS OF *USTILAGO ZEAE*¹

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INTRODUCTION

Extensive corn-breeding investigations at different places in the United States have shown that selfed lines of corn resistant to *Ustilago zeae* (Beckm.) Ung. are rather readily isolated by inbreeding and selection (3, 6, 13).³ The importance of developing smut-resistant varieties of corn is obvious, since the smut cannot be controlled satisfactorily by seed treatment or by cultural practice.

Recent investigations have emphasized the important role that physiologic specialization plays in the development of smut-resistant varieties of cereal crops. However, the significance of physiologic specialization in breeding smut-resistant varieties of corn is not known. Christensen and Stakman (2) and Eddins (3) have demonstrated that varieties and selfed lines of corn react differently to different collections of smut when inoculated by artificial methods. Recent studies have shown that *Ustilago zeae* comprises an indefinite number of lines or biotypes, some of which frequently give rise to variants in culture (1, 15). Furthermore, it has been demonstrated that *Ustilago zeae* is predominantly heterothallic, usually requiring the pairing of two unisexual lines of opposite sex for normal infection and production of chlamydospores (1, 14). There is, however, only meager information on the behavior of varieties and selfed lines of corn under field conditions when inoculated with a mixture of chlamydospores of *Ustilago zeae* from different localities. A preliminary study in Minnesota by Inner and Christensen, however, indicated that selfed lines of corn did not react differently to smut collections from University Farm and those collected in different localities throughout the Mississippi Basin (10).

This paper reports two additional years' data on the collections of smut previously used and data on collections obtained from a wider area.

MATERIAL AND METHODS

A representative group of approximately 100 selfed lines of corn of all gradations for resistance and susceptible to smut were selected from field, sweet, and pop-corn types. Five standard varieties of corn also were included in the tests. The same selfed lines of corn

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³ Reference is made by number (*italic*) to Literature Cited, p. 56.

were used throughout the tests, thus giving opportunity for studying seasonal fluctuations in severity of infection and the degree of constancy of reaction of these lines from year to year. Thirty plants of all lines were grown in each of two replications on each of two fields designated E and X, and separated by more than a mile. On field E an artificial smut of epiphytotic was induced by using chlamydospores collected from University Farm only; and on field X, from 1928-31, inclusive, a similar epiphytotic was induced by using a mixture of smut collected from numerous localities in the northern Mississippi Valley. In 1932 the study on field X was discontinued, and similar inoculations were made on field T, but additional collections of smut were obtained from 26 localities in 12 States east of the Rocky Mountains. In the fall smut galls were gathered from infected plants in each field and used for inoculum during the following year on the same fields. The epiphytotic was induced each year by mixing a portion of the chlamydospores with fresh barnyard manure before the manure was applied in the rows when the corn plants were from 12 to 18 inches tall. At frequent intervals during the growing season chlamydospores also were sprayed or dusted on the plants. When the corn was almost mature notes were taken on the number of infected plants and on the size, location, and number of the smut galls per plant.

EXPERIMENTAL RESULTS

The behavior of selfed lines and varieties of corn to local and non-local collections of *Ustilago zeae* was determined by analysis of the total percentage of smut infection, location of galls, and number and size of galls per plant.

REACTIONS OF CORN TO SMUT

The reaction of 20 representatives of 95 selfed lines of corn grown for 6 years and inoculated with chlamydospores of *Ustilago zeae* of local and nonlocal origin is given in table 1. The results indicate that most of the selfed lines used in this study were relatively homozygous for smut reaction. In general, the lines which were resistant 1 year tended to be resistant the following year, while lines susceptible 1 year proved to be susceptible the following year. The interannual correlation coefficients on the reaction of all the lines of corn grown in field E between 1930 and 1931 was 0.683; between 1931 and 1932, 0.684; and between 1932 and 1933, 0.700. These coefficients all exceed the 1-percent point (odds greater than 99:1) in significance and add further evidence of the relative homozygosity of the lines of corn to specific smut reaction.

The data presented in table 1 indicate also that selfed lines in a given year had a tendency to react alike on fields E and X or T. In all years except 1931 the average smut percentage was slightly higher on field E than on the other two fields. The differences, however, are not statistically significant. The reaction of all lines to the two collections of smut was determined by comparing the correlation coefficient between two replicates on the same field with the correlation coefficient between single replicates of the two fields. These results are summarized in table 2. Table 2 also includes the 2-year summary of the study made in 1928 and 1929, which was previously reported by Immer and Christensen (10).

TABLE 1.—*Smut reaction of 20 selfed lines of corn inoculated with chlamydo-spores of local and nonlocal origin, 1928-33, grown on three different fields*

Culture no.	Years selfed	Percentage of smut															
		1928		1929		1930		1931		1932		1933		Average			
		Field E ¹	Field X	Field E	Field X	Field E	Field X	Field E	Field X	Field E	Field T	Field E	Field T	Field E	Fields X and T		
		Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent		
B-12	8	4	0	7	0	11	0	19	20	17	5	25	17	14	7		
Z-16	9	23	5	56	22	56	39	32	33	67	33	70	57	51	32		
Z-17	9	35	10	2	4	15	4	8	15	10	0			14	8		
Z-24	6	2	0	5	2	9	0	14	11	15	11	21	14	11	6		
Z-28	9	15	14	38	42	51	41	35	23	63	44	40	41	40	34		
Z-32	11	23	0	21	2	5	9	44	24	13	18			21	11		
Z-41	9	9		30	47	38	31	38	40	21	12	53	7	36	27		
Z-42	8	1	0	4	0	3	0	4	2	4	3	5	0	4	1		
Z-43	10	8		20	6	25	18	24	48	55	46	42	43	20	28		
Z-54	7	8		2	2	0	0	2	2	0	8	7	4	2	3		
Z-58	8	8		70	84	81	38	74	90	81	86	76	84	78	76		
Z-73	7	3	7	4	0	2	4	2	2	0	3	2	3	2	3		
Z-75	7	10	0	18	2	4	4	13	20	6	8	13	17	11	9		
Z-76	7	13	6	15	2	10	16	15	9	19	29			14	12		
Z-78	8	12	0	18	0	8	3	31	13	7	14	12	38	15	11		
Z-80	7	9	6	23	14	48	38	37	42	39	64	35	50	32	36		
Z-85	7			99	77	50	86	20	8	17	21	11	5	39	36		
Z-88	8	0	0	47	32	31	22	92	63	70	52	44	48	47	36		
Z-93	8	9	0	17	8	19	5	39	55	30	4			23	14		
Z-100	8	22	7	24	21	2	0	17	32	25	33	31	39	20	22		
Average		12	4	26	18	23	18	28	28	28	25	30	29	25	21		

¹ Field E was inoculated with chlamydospores of *Ustilago zeae* collected at University Farm, Minn.; field X was inoculated with chlamydospores obtained from the Mississippi Valley region. In 1932 field X was discontinued and similar inoculations were made on field T, but additional chlamydospore collections were obtained from wider sources.

TABLE 2.—*Correlation coefficients between percentage of smut infection of selfed lines of corn and varieties grown in two replicates in fields E and X or T, 1928-33*

Nature of correlation	Correlation coefficients					
	1928	1929	1930	1931	1932	1933
First and second replicate of field E	0.599±0.02	0.648±0.023	0.776±0.027	0.715±0.036	0.768±0.02	0.726±0.033
First and second replicate of field X	.595±.023	.655±.023	.810±.02	.664±.041	.694±.036	.838±.021
Average	.597±.024	.651±.023	.794±.027	.630±.033	.733±.033	.788±.027
First replicate of field E with first replicate of field X	.350±.031	.624±.024	.619±.04	.720±.036	.673±.035	.775±.027
Second replicate of field E with second replicate of field X	.388±.030	.676±.024	.779±.027	.607±.046	.624±.04	.781±.027
Average	.374±.031	.653±.023	.709±.034	.668±.039	.652±.040	.778±.027
Average infection field E with average infection field X or T	.473±.028	.748±.018	.759±.029	.813±.037	.850±.020	.854±.019
N	358	281	100	84	92	93

An analysis of the data in table 2 clearly shows that, with the exception of the 1928 season, the correlations between two replicates within a field are essentially the same as the correlations of single replicates between the fields E and X. The average coefficients given

in table 2 and other tables were obtained by the method given by Fisher (4, p. 164). The average correlation coefficients between two replicates within a field were not significantly different from the average correlation coefficient of single replicates between the fields from 1929 to 1931, inclusive. These results indicate that, on an average, the lines used reacted similarly to the local and nonlocal smut collections. In 1928 there was a lower correlation between individual series of two fields as compared with the correlation between series of the same field. Immer and Christensen (10), however, report that the seed planted in 1928 on field X germinated 2 weeks later than that on field E, because of unfavorable conditions on the former field, and suggested this as the possible cause of differential response. The present data tend to support this conclusion.

TABLE 3.—Comparison of 5- or 6-year average smut infection on 20 resistant lines of corn inoculated with local and nonlocal smut collections and grown on three different fields

Culture no.	Years selfed	Years averaged	Percentage of infection		Increase (+) or decrease (-) in per- centage of infection in fields X and T over that in field E
			Field E	Fields X and T	
	Number	Number	Percent	Percent	Percent
S-73.....	7	6	2.1	3.2	+1.1
S-54.....	7	5	2.8	2.6	-0.2
S-42.....	8	6	3.4	1.1	-2.3
S-9.....	8	5	3.5	4.5	+1.0
S-31.....	10	5	3.6	1.0	-2.6
S-15.....	8	6	4.0	5.8	-0.9
S-77.....	7	6	5.1	6.8	+1.7
S-56.....	7	5	7.2	9.9	+2.7
S-35.....	9	5	8.2	8.2	0.0
S-75.....	7	6	8.8	12.5	+3.7
S-85.....	7	6	9.1	10.9	+1.8
S-34.....	6	6	10.9	6.1	-4.8
S-84.....	7	6	11.2	11.3	+0.1
S-64.....	8	5	11.6	7.7	-3.9
S-72.....	7	5	13.1	10.6	-2.5
S-98.....	8	6	13.5	13.2	-0.3
S-78.....	8	6	14.4	11.4	-3.0
S-90.....	7	6	17.9	14.7	-3.2
S-26.....	10	5	18.3	22.7	+4.4
S-82.....	8	6	19.5	8.5	-11.0

Since the conclusions drawn from a study of the correlation coefficients in table 2 are based on the average reaction of a large number of lines rather than on individual lines, a further study of the data was made to determine whether there were significant differences between the reaction of individual resistant lines grown on the two fields on which local and nonlocal smut had been applied. The data of these comparisons for the 5- to 6-year period are summarized in table 3. In general, the selfed lines of corn resistant to local collections of smut on field E were equally resistant in fields X or T inoculated with a mixture of smut from many different localities. None of the differences in smut reaction between the lines listed in table 3 are statistically significant. In order to make more direct comparisons with the two different collections, resistant lines were analyzed on the basis of the periods in which the lines on field X and T were inoculated. During the 4-year period 1928 to 1931, inclusive, 26 lines, with an average

smut reaction from 1 to 10 percent, grown on field E were compared with the same lines grown on field X. None of these lines of corn had a significant higher average smut reaction when grown on field X than when grown on field E. During this period the smut reaction of another group of 26 lines, with average smut infection from 10 to 20 percent on field E, were similarly compared with their smut reaction on field X. Likewise, none of these lines reacted significantly differently on field X.

In a similar manner the smut reaction of 16 selfed lines of corn that averaged less than 10 percent of smut when grown on field E were compared with their reactions on field T in 1932 and 1933. None of these lines developed a significantly higher percentage of smut when inoculated with smut of nonlocal origin than when inoculated with the smut from University Farm collections. The maximum deviation in smut infection for a particular line occurred in 1933, when line S-78 developed 26.6 percent more smut on field T than on field E. However, the difference for the 2-year average was only 17 percent, which was 1.5 times the probable error. For the 5-year period (1929-33) the average infection on line S-78 was 3 percent more on the field E inoculated with local smut than on fields X and T inoculated with nonlocal smut.

The 2-year average smut infection during the same period of another group of 20 lines, in which the infection ranged between 10 and 20 percent on field E, was compared with their reaction on field T. There were no statistically significant differences in infection between the same lines on the two separate fields. However, in a few cases there were considerable differences in percentages of smut infection in the same line grown on fields E and T. For example, during 1932 and 1933, line S-79 developed on an average 25.5 ± 13.5 percent more smut in field T than in field X. A comparison of the specific location of the smut galls on this line in each field showed that in 1932 the increase in infection on field T was due to a greater number of infections on the suckers, and in 1933 the increase was due to a greater number of ear infections on field T. In general these results agree with those obtained previously by Immer and Christensen (10).

The effect of variation in environmental conditions on the prevalence of corn smut has been recognized (9). Recently, Walter⁴ has accounted for fluctuation in susceptibility within a line or variety on the basis of the interaction of the growth phase of the host and environment.

The data on 61 selfed lines of corn grown on field E, X, or T from 1929 to 1933, inclusive, were used to determine the extent of variability in average smut infection that was dependent on seasonal fluctuation or on differences in reaction between fields. In 1929 and 1930 there was significantly less smut on the selfed lines grown on field X than on field E (table 4). For these 2 years there was, respectively, 11.2 ± 2.39 and 7.9 ± 2.23 percent less smut on field X than on field E, inoculated only with the smut from University Farm. In the next 3 years (1931-33) there were no significant differences in percentages of smut infection in the fields inoculated with local and nonlocal smut collections. There were also significant interannual differences in percentages of infection between years on field X but not on field E or

⁴WALTER, J. M. SOME FACTORS INFLUENCING THE DEVELOPMENT OF CORN SMUT, *USTILAGO ZEA* (BECKM.) UNG. 87 pp., illus. (Unpublished doctor's thesis, Univ. Minn.)

T. For instance, the difference between the average infection on field X for years 1930 and 1931 was 8.4 ± 2.14 , but for field E it was only 3.1 ± 2.48 . It is, however, worthy of note that the relative susceptibility among selfed lines of corn was not materially altered, despite differences in severity of infection between fields (tables 1 and 3). Nevertheless, the results indicate that for the 6-year period (1928-33), growth and environmental factors were more instrumental in influencing the reaction of a line of corn to smut than were the different collections of smut employed. Therefore, to eliminate error due to growth and environmental conditions, comparative pathogenicity tests should be made under as nearly identical conditions as possible and over a period of years.

TABLE 4.—Average percentage of smut infection on 61 selfed lines of corn grown on field E, X, or T, 1929-33

Year	Total smut percentage on fields indicated		Increase (+) or decrease (-) in percentage of infection in field E over that in fields X or T	Year	Total smut percentage on fields indicated		Increase (+) or decrease (-) in percentage of infection in field E over that in fields X or T
	Field E	Field X or T			Field E	Field X or T	
	Percent	Percent	Percent		Percent	Percent	Percent
1929..	25.9 \pm 1.88	14.7 \pm 1.48	11.2 \pm 2.39	1932..	29.6 \pm 1.71	28.5 \pm 1.57	1.1 \pm 2.34
1930..	25.0 \pm 1.75	17.1 \pm 1.39	7.9 \pm 2.23	1933..	30.2 \pm 1.72	30.1 \pm 2.00	1.1 \pm 2.64
1931..	28.1 \pm 1.76	25.5 \pm 1.63	2.6 \pm 2.40				

SPECIFIC LOCATION OF SMUT GALLS

Several investigators (5, 6, 7, 9, 10) have found that some selfed lines of corn have a tendency to become infected on definite parts of the plant, i. e., tassel, neck (peduncle), or ear. The reason for such specificity in location of smut galls is not definitely known, although it has been suggested that physiologic forms may be partly responsible. Immer (8) concluded that the position of the gall most likely was associated with certain morphological characters. During 1932 and 1933 observations were made on the relative frequency of galls on ears, suckers,⁵ and shoots (potential ear buds) on 84 lines of corn grown on fields E and T. These locations were selected in preference to others because of the relative frequency of gall occurrence on them. The behavior of 16 selfed lines (representative of 84 lines) inoculated with chlamydospores of local and nonlocal origin is given in table 5. The results indicate clearly that the 16 lines of corn in the given years reacted similarly with respect to specificity of position of galls on plants in the two fields. There was a greater difference between percentage of infection in specific locations in the same fields in different years than between different fields in the same year. (See cultures S-3 and S-7 in table 5.)

Statistical analysis of the data in table 6 shows that the correlation coefficients of infection of 84 lines at specific locations between 1932 and 1933 in the same fields (E and T) were no greater than between different fields. These results indicate that the different lines of corn had a tendency to become infected at the same location, whether inoculated with local or nonlocal collections of chlamydospores. The

⁵ Sucker smut-galls located on any part of a stalk arising from the basal nodes.

results further indicate that the tendency for specificity of location of galls is characteristic of the lines of corn concerned.

NUMBER AND SIZE OF GALLS PER PLANT

In a similar manner, a study was made of the number and size of galls per plant on 84 selfed lines of corn grown on fields E and T. The data of this study are presented in tables 7 and 8. The correlation coefficients obtained for number of galls per infected plant on the same fields for different years were approximately of the same magnitude as those for the two fields in the same year. Apparently the different smut collections did not influence the number of smut galls that developed per infected plant.

TABLE 5.—Comparison of the percentages of smut on 16 selfed lines of corn with infection at specific locations when grown on fields E¹ and T in 1932 and 1933

Culture No	Years selfed	Percentage of smut on—											
		Ear				Sucker				Shoot			
		1932		1933		1932		1933		1932		1933	
		Field E	Field T	Field E	Field T	Field E	Field T	Field E	Field T	Field E	Field T	Field E	Field T
		Num- ber	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent
3	9	7	5	50	42	0	2	0	2	20	39	23	37
7	8	0	2	28	17	3	4	8	7	16	7	14	5
10	8	0	0	0	0	5	0	0	0	0	0	6	16
14	16	0	0	13	15	32	17	22	8	5	22	19	4
17	9	0	0	2	0	0	2	2	2	0	0	0	0
25	6	10	10	12	21	37	17	14	23	22	24	20	17
30	11	6	2	20	35	72	36	26	45	6	2	5	30
38	8	11	9	34	57	0	0	0	4	19	26	43	57
58	8	27	29	52	64	54	39	24	48	35	25	19	24
66	8	2	0	2	0	25	27	2	15	16	13	4	4
71	8	0	0	0	0	0	0	0	0	22	16	2	11
88	8	2	2	0	3	2	9	2	8	66	24	40	28
89	8	0	0	2	0	6	10	14	8	9	13	2	3
92	8	0	0	0	0	33	30	11	3	1	0	0	0
97	7	0	0	2	0	5	4	0	5	25	11	25	19
100	8	0	0	10	8	4	0	0	2	15	25	23	29

¹ Field E was inoculated with chlamydozoospores of *Ustilago zeae* collected at University Farm, Minn., while field T was inoculated with chlamydozoospores obtained from many different localities east of the Rocky Mountains

TABLE 6.—Annual and interannual correlations of the location of smut galls on 84 selfed lines of corn grown on fields E¹ and T in 1932 and 1933

Nature of correlation	Correlation coefficients at specific location of infection indicated		
	Shoot	Sucker	Ear
Field E, 1932 and 1933	0.741±0.033	0.645±0.043	0.596±0.047
Field T, 1932 and 1933	.564±.050	.677±.041	.800±.027
Average	.662±.041	.661±.041	.713±.036
Fields E and T, 1932	.670±.041	.709±.030	.760±.031
Fields E and T, 1933	.698±.038	.731±.034	.799±.027
Average	.684±.039	.751±.032	.781±.029

¹ See footnote 1, table 5.

TABLE 7.—Average number of smut galls per infected plant and average size of galls on selfed lines of corn grown on fields E¹ and T in 1932 and 1933

Culture No.	Years selfed	Galls per infected plant ²				Average size ³ of galls ²			
		Field E		Field T		Field E		Field T	
		1932	1933	1932	1933	1932	1933	1932	1933
	Number	Number	Number	Number	Number				
S-2	7	1.00	1.00	1.00	1.00	1.60	1.00	1.50	1.25
S-7	7	1.21	1.59	1.85	1.20	1.41	1.98	1.41	1.77
S-10	8	1.00	1.19	1.00	1.05	1.50	1.36	1.00	1.35
S-13	8	1.15	1.25	1.00	1.27	1.80	1.48	1.44	1.45
S-14	16	1.00	1.50	1.06	1.20	1.58	1.92	1.72	1.92
S-16	8	1.14	1.54	1.23	1.03	1.83	1.98	1.76	2.03
S-18	8	1.18	1.75	1.14	1.60	1.92	1.79	1.75	1.25
S-24	6	1.00	1.11	1.00	1.14	1.42	1.10	1.50	1.38
S-25	9	1.38	1.61	1.53	1.78	1.84	1.58	1.67	1.45
S-27	8	1.00	1.60	1.00	1.44	1.50	1.59	1.50	1.17
S-28	8	1.63	1.41	1.13	1.33	1.69	1.38	1.59	1.36
S-30	11	1.33	1.18	1.16	2.39	1.98	2.08	1.65	1.68
S-34	9	1.24	1.60	1.23	1.80	1.67	1.92	1.63	1.41
S-62	8	1.11	1.20	1.14	1.11	1.39	1.39	1.52	1.10
S-67	8	1.17	1.59	1.18	1.79	1.67	1.39	1.50	1.34
S-80	7	1.28	1.57	1.09	1.29	1.65	1.27	1.64	1.22
S-85	7	1.00	1.14	1.09	1.00	1.44	1.25	1.17	1.00
S-98	8	1.20	1.17	1.00	1.00	1.17	1.43	1.80	1.20

¹ See footnote 1, table 5² Based on average of 2 replicates³ Smut galls are divided into 3 classes 1=galls less than 2 inches in any diameter, 2=galls between 2 and 4 inches in diameter, 3=galls 4 inches or more in diameter.TABLE 8.—Annual and interannual correlation on the number of smut galls per infected plant and average size of galls on 84 selfed lines of corn grown on fields E¹ and T in 1932 and 1933

Nature of correlation ²	Galls per plant	Average size ³ of galls
Field E, 1932 and 1933	0.431±0.0599	0.208±0.0704
Field T, 1932 and 1933	401± .0618	.002± .0736
Average	417± .0608	.104± .0728
Fields E and T, 1932.	.461± .0580	.117± .0726
Fields E and T, 1933.	.573± .0494	.507± .0474
Average	.519± .0538	.277± .0679

¹ See footnote 1 table 5² Based on 2 replicates in each field³ Gall size 1=galls less than 2 inches in any diameter, 2=galls between 2 or 4 inches in any diameter, 3=galls 4 inches or more in any diameter.

It is evident from tables 7 and 8 that the size of galls per infected plant is of little value in making comparative studies of susceptibility between lines of corn, and consequently also between collections of smut. There was a fairly high correlation between percentage of smut infection and number of galls per infected plant, but there was no correlation between percentage of smut infection and size of gall. Thus a correlation coefficient between the average 2-year percentage of smut infection and number of galls per plant for field E was 0.672 ± 0.060 and for field T, 0.750 ± 0.042 ; which indicates that susceptible lines tend to have more galls per plant than resistant lines. The correlation coefficients between average size of smut galls and percentage of smut infection obtained for field E was 0.044 and for field T, 0.013.

DISCUSSION

The results of these comparative studies on the response of selfed lines of corn to mass inoculation with chlamydospores of *Ustilago zeae* from different localities in the United States under natural field conditions are of particular value in the application to breeding for smut resistance. It was clearly shown that lines of corn resistant to local collections of smut were resistant to smut collections from widely different sources. The fact that there is close agreement in smut reaction of lines of corn to smut collections from many different localities should greatly facilitate the breeding of corn for smut resistance. In fact, some progress has been made. A number of promising crosses produced from resistant or semiresistant lines of corn have proven to be more resistant than the original varieties when subjected to a similar epiphytotic of *U. zeae* obtained from widely different sources.

Christensen and Stakman (2), Immer (8), Immer and Christensen (9), Hoover (7), Walter,⁶ Kyle (11), and others (3, 10, 12) have demonstrated that there are physiological, morphological, and perhaps also functional types of resistance in corn to *Ustilago zeae*. The failure of selfed lines of corn to respond in a differential manner to different collections of smut indicates that the resistant lines used in these tests possess some type of resistance to the pathogen other than the physiologic. Obviously the lines of corn tested have been exposed to infection by numerous biotypes of *Ustilago zeae*. This has been proven by numerous isolation tests. Since field E and neighboring fields have been planted to corn for many years, it is highly probable that new biotypes of smut arising from hybridization, mutation, or introduction, have had ample opportunity to persist. It seems likely, therefore, that lines of corn physiologically susceptible did not escape infection. This evidently accounts for the similarity in behavior of the selfed lines in both fields.

It has been shown by the writers and others that environmental factors influence the prevalence of smut on varieties and selfed lines of corn. It is, however, highly significant that the relative susceptibility of the particular lines tested remained constant from year to year, although the percentages of infection differed considerably. Therefore, selfed lines or crosses which are resistant at this station could probably be utilized over a fairly wide area with some assurance that they would be resistant in regions with similar climatic conditions.

To insure selection of smut-resistant lines of corn it would seem highly desirable to subject them for a period of years to as severe epidemics of smut as possible. Furthermore, in order to eliminate as far as possible physiologically susceptible lines the inoculum should be obtained from the general region in which the corn is to be grown.

SUMMARY

A study was made of the behavior of 5 varieties and 95 selfed lines of corn under natural field conditions when inoculated with smut collections of chlamydospores from University Farm, St. Paul, Minn., and collections obtained from numerous localities in 12 different States east of the Rocky Mountains.

The annual correlation coefficients between percentages of smut infection in replicates of each field with local and nonlocal smut,

⁶ Walter, J. M. See footnote 4.

respectively, were essentially of the same magnitude as those between single replicates of the different fields inoculated. In general, these results indicate that the relative reactions of all the lines of corn were similar on the two fields.

Comparison of the average smut reactions of individual resistant lines grown on two fields failed to show a single instance of statistically significant difference. Lines of corn resistant to local smut collections were equally resistant to smut obtained from widely different sources east of the Rocky Mountains.

Growth and environmental factors caused greater fluctuation in prevalence of smut than did the different collections of smut, but the relative susceptibility of selfed lines of corn was not altered.

A study was made in 1932 and 1933 of smut infections at particular locations on the plant (ear, sucker, and shoot). No significant differences were found between smut reactions in different years in the same field and reactions in the same year in different fields. This indicates that lines of corn had a tendency to become infected at definite locations irrespective of collections of smut used.

In a similar manner it was shown that the different smut collections did not influence the number of smut galls that developed per infected plant. There was a high correlation between number of galls per infected plant and percentage of smut infection.

The size of smut galls cannot be used as the sole measure of resistance or susceptibility.

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PROCEDURE FOR RAPID CALCULATION OF MULTIPLE CORRELATION COEFFICIENTS¹

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INTRODUCTION

The use of calculating machines has enabled investigators to enlarge and expand their work far beyond what would otherwise have been practicable. Those who use correlation coefficients have undoubtedly benefited greatly by the modern calculators. However, even the machine calculation of multiple correlation is a long, laborious procedure.

In using Wallace and Snedecor's machine methods² the writer has devised some short cuts which not only save a great deal of time and labor but give to the calculations a greater degree of accuracy.

The present paper reports these simplified methods of obtaining multiple correlations by the modern electric calculator.

CALCULATING THE MULTIPLE CORRELATION COEFFICIENT

Wallace and Snedecor³ have given in detail the method for solving the normal equations necessary for calculating the multiple correlation coefficient. Ezekiel⁴ also has given such a method. It is in these methods of solving the normal equations that the writer's short cuts are found.

In each of tables 1 to 9 progressively are shown the necessary figures or numbers from which to calculate the multiple correlation coefficient. The nine tables give a full set of numbers for the calculation of all nine multiple correlation coefficients.

¹ Received for publication Sept. 7, 1934; issued April, 1935

² WALLACE, H. A., and SNEDECOR, G. W. CORRELATION AND MACHINE CALCULATION. Rev. by G. W. Snedecor. 71 pp., illus. Ames. 1931 (Iowa State Col. Off. Pub. 30, no. 4).

³ WALLACE, H. A., and SNEDECOR, G. W. See footnote 2

⁴ EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London. 1930.

TABLE 1.—The necessary numbers and products of the calculations to the completion of the "I" multiple correlation coefficient where nine characters are involved

	A	B	C	D	E	F	G	H	I	S	Accuracy
A.....	1 0000	0 0243	0 0590	-0.0306	0 2236	0.4580	0 2941	0 1265	0 1554	2.3113	
B.....		- 0243	- 0590	.0306	- 2236	- 4580	- 2941	- 1265	- 1554	2.4653	
C.....		1 0000	.6264	.5754	.0528	- 0282	- 1025	.3251	- 0080	2.4691	
D.....		-1.0000	.6250	.5761	.0474	- 0394	- 1096	.3220	.0118	-2.4106	+1
E.....			- 0253	- .5765	- .0474	.0394	.1097	- 3222	.0118	-2.4106	
F.....			1.0000	.6294	.1994	- 3173	- 2848	.4446	.1399	2.4966	
G.....			.6087	.2710	.1599	- 3197	- 2336	.2358	.1381	.8538	
H.....			-1.0000	- 4474	- 2585	.5279	.3857	- 3893	- 2280	- 1.4096	(1)
I.....				1 0000	- 1903	- 1735	- 1574	- 1953	- 1781	1.6702	
D.....				.5457	- 2808	.0903	.0193	- 0920	- 2283	- 0299	
E.....				1 0000	.6147	- 0115	- 0354	.1685	.4184	.0548	+1
F.....					1 0000	- 0457	.0636	.3741	.0848	2.3623	
G.....					.7627	- 0606	.0734	.2222	.4974	1.4952	
H.....					1 0000	.0794	- .0962	- 2014	.6522	- 1.9604	(1)
I.....						1 0000	.7321	- 1767	- .0806	1.3594	
F.....						.6141	.4751	- 0789	- .0474	.9632	
G.....						- 7741	.1285	.0771	- 1.5985	(1)	
H.....							1 0000	- 1475	.1456	1.5435	
I.....							.4356	- 0155	.1488	.5689	
H.....							1 0000	.0355	.3416	- 1.4061	(1)
I.....								1 0000	.4270	2.5684	
H.....								.6975	.1732	.8706	
I.....								1 0000	- 2483	- 1.2482	- 1
Beta..	1A + 0459	1B - 0337	1C + 0172	1D - 1172	1E + 5210	1F + 3164	1G + 3504	1H + 2483			

1 Checked to fourth place exactly

$$R = \sqrt{0.57317101} = 0.7571$$

TABLE 2.—The necessary numbers and products, in addition to those found in table 1, to complete the calculation of the "H" multiple correlation coefficient where nine characters are involved

	A	B	C	D	E	F	G	I	H	S	Accuracy
I								1 0000 4098 -1 0000	0 4270 1732 -3687	2 2760 6126 -1 3083	(-4)
Beta	HA { + 0737	HB + 1497	HC + 2296	HD + 0264	HE + 0684	HF + 0249	HG - 1611	HI + 3687			

$$R = \sqrt{0.30632281} = 0.6052$$

TABLE 3.—The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "G" multiple correlation coefficient where nine characters are involved

	A	B	C	D	E	F	I	H	G	S	Accuracy
I.....							1 0000	0 4270	0.1156		
H.....							.5206	.1679	.1488	0 8372	- 2
Beta..	GA - 0890	GB - 0817	GC - 0590	GD + 1324	GE - 0198	GF + 7859	GI + 3176	GH - 0986			

$$R = \sqrt{0.61310627} = 0.7830$$

TABLE 4.—The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "F" multiple correlation coefficient where nine characters are involved

	A	B	C	D	E	I	H	G	F	S	Accu- racy
I						$\left\{ \begin{array}{l} 1.0000 \\ .5243 \\ -1.0000 \end{array} \right.$	$\left\{ \begin{array}{l} 0.4270 \\ .1740 \\ -.3319 \end{array} \right.$	$\left\{ \begin{array}{l} 0.1456 \\ .1121 \\ -.2138 \end{array} \right.$	$\left\{ \begin{array}{l} -.0906 \\ -.0474 \\ .0904 \end{array} \right.$	$\left\{ \begin{array}{l} 0.7629 \\ 0.7629 \\ -1.4551 \end{array} \right.$	-2
H							$\left\{ \begin{array}{l} 1.0000 \\ .708201 \\ .6505 \\ -1.0000 \end{array} \right.$	$\left\{ \begin{array}{l} -.1475 \\ -.076551 \\ -.1138 \\ .1749 \end{array} \right.$	$\left\{ \begin{array}{l} -.1787 \\ -.0789 \\ -.0632 \\ .0971 \end{array} \right.$	$\left\{ \begin{array}{l} .726685 \\ .4735 \\ -.7279 \end{array} \right.$	-1
G								$\left\{ \begin{array}{l} 1.0000 \\ .803638 \\ .7598 \\ -1.0000 \end{array} \right.$	$\left\{ \begin{array}{l} .7324 \\ .4754 \\ .4745 \\ -.6245 \end{array} \right.$	$\left\{ \begin{array}{l} 1.314556 \\ 1.2343 \\ -1.6245 \end{array} \right.$	(1)
Beta	$\left\{ \begin{array}{l} FA \\ + 3115 \end{array} \right.$	$\left\{ \begin{array}{l} FB \\ + 2030 \end{array} \right.$	$\left\{ \begin{array}{l} FC \\ - 1960 \end{array} \right.$	$\left\{ \begin{array}{l} FD \\ - 1010 \end{array} \right.$	$\left\{ \begin{array}{l} FE \\ + 0056 \end{array} \right.$	$\left\{ \begin{array}{l} FI \\ - 2279 \end{array} \right.$	$\left\{ \begin{array}{l} FH \\ + 0121 \end{array} \right.$	$\left\{ \begin{array}{l} FG \\ + 6245 \end{array} \right.$			

¹ Checked to fourth place exactly

$R = \sqrt{0.69260575} = 0.8322$

TABLE 5. The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "E" multiple correlation coefficient where nine characters are involved

	A	B	C	D	I	H	G	F	E	S	Accu- racy
I					$\left\{ \begin{array}{l} 1.0000 \\ .8487 \\ -1.0000 \end{array} \right.$	$\left\{ \begin{array}{l} 0.4270 \\ .3189 \\ -.3758 \end{array} \right.$	$\left\{ \begin{array}{l} 0.1456 \\ .1599 \\ -.1884 \end{array} \right.$	$\left\{ \begin{array}{l} -.0906 \\ -.0869 \\ .1024 \end{array} \right.$	$\left\{ \begin{array}{l} 0.0848 \\ .4974 \\ -.5861 \end{array} \right.$	$\left\{ \begin{array}{l} 1.7381 \\ 1.7381 \\ -2.0490 \end{array} \right.$	+1
H						$\left\{ \begin{array}{l} 1.0000 \\ .772950 \\ .6531 \\ -1.0000 \end{array} \right.$	$\left\{ \begin{array}{l} -.1475 \\ -.055175 \\ -.1153 \\ .1765 \end{array} \right.$	$\left\{ \begin{array}{l} -.1767 \\ -.096540 \\ .0639 \\ .0978 \end{array} \right.$	$\left\{ \begin{array}{l} .3741 \\ .2222 \\ .0353 \\ -.0540 \end{array} \right.$	$\left\{ \begin{array}{l} 1.162386 \\ 1.162386 \\ .5092 \\ -.7797 \end{array} \right.$	(1)
G							$\left\{ \begin{array}{l} 1.0000 \\ .810699 \\ .7602 \\ -1.0000 \end{array} \right.$	$\left\{ \begin{array}{l} .7324 \\ .469550 \\ .4746 \\ -.6244 \end{array} \right.$	$\left\{ \begin{array}{l} .0636 \\ .0734 \\ .0141 \\ .0185 \end{array} \right.$	$\left\{ \begin{array}{l} 1.458394 \\ 1.2208 \\ -1.6059 \end{array} \right.$	(1)
F								$\left\{ \begin{array}{l} 1.0000 \\ .618925 \\ .3074 \\ 1.0000 \end{array} \right.$	$\left\{ \begin{array}{l} -.0457 \\ -.0806 \\ .0026 \\ -.0084 \end{array} \right.$	$\left\{ \begin{array}{l} .844497 \\ 3100 \\ -1.0085 \end{array} \right.$	+1
Beta	$\left\{ \begin{array}{l} FA \\ + 1089 \end{array} \right.$	$\left\{ \begin{array}{l} FB \\ + 0497 \end{array} \right.$	$\left\{ \begin{array}{l} FC \\ + 2226 \end{array} \right.$	$\left\{ \begin{array}{l} FD \\ - 2659 \end{array} \right.$	$\left\{ \begin{array}{l} EI \\ + 5724 \end{array} \right.$	$\left\{ \begin{array}{l} EH \\ + .0506 \end{array} \right.$	$\left\{ \begin{array}{l} EG \\ - 0237 \end{array} \right.$	$\left\{ \begin{array}{l} EF \\ + 0084 \end{array} \right.$			

¹ Checked to fourth place exactly

$R = \sqrt{.53097919} = 0.7287$

TABLE 6.—The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "D" multiple correlation coefficient where nine characters are involved

	A	B	C	I	H	G	F	E	D	S	Accuracy
I.....				1.0000 .9442 -1.0000	0.4270 .3574 -3785	0.1456 .1519 -1609	-0.0906 -.0895 .0948	0.6848 .6149 -6512	-0.1781 -.2283 .2418	1.7506 -1.8541	+1
H.....				1.0000 .788452 .6532 -1.0000	0.4270 -1475 -1159 1775	0.1456 -058432 -1159 1775	-0.0906 -.097508 -.0637 .0676	0.6848 .298597 .0369 -0564	-0.1781 -.0920 -.0050 -.0080	1.167424 .5048 -7728	+1
G.....				1.0000 .811383 7964 -1.0000	0.4270 -1475 -1159 1775	0.1456 -058432 -1159 1775	-0.0906 -.097508 -.0637 .0676	0.6848 .298597 .0369 -0564	-0.1781 -.0920 -.0050 -.0080	1.457336 1.2653 -1.6509	-1
F.....				1.0000 .618097 .3125 -1.0000	0.4270 -1475 -1159 1775	0.1456 -058432 -1159 1775	-0.0906 -.097508 -.0637 .0676	0.6848 .298597 .0369 -0564	-0.1781 -.0920 -.0050 -.0080	.844153 .2787 -8918	+1
E.....				1.0000 .907275 .5029 -1.0000	0.4270 -1475 -1159 1775	0.1456 -058432 -1159 1775	-0.0906 -.097508 -.0637 .0676	0.6848 .298597 .0369 -0564	-0.1781 -.0920 -.0050 -.0080	1.510595 .3757 -7471	(1)
Beta.....	DA (+ 0281	DB + 2700	DC + 5150	DI - .1226	DH + 0185	DG + 1526	DF - 1465	DE - .2529			

1 Checked to fourth place exactly.

$$R = \sqrt{.55361263} = 0.7441$$

TABLE 7.—The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "C" multiple correlation coefficient where nine characters are involved

	A	B	I	H	G	F	E	D	C	S	Accuracy
I.....			1.0000 .9757 -1.0000	0.4270 4111 -4213	0.1456 0986 -1624	-0.0906 -.1624 .6590	0.6848 -.1665 -1706	-0.1781 -.1665 .1706	0.1399 1381 -1415	1.9453 -1.9937	(1)
H.....			1.0000 .880249 7071 -1.0000	0.4270 -1475 -1909 2700	0.1456 -1475 -1909 2700	-0.0906 -.1767 -.0565 -.0798	0.6848 .3741 .013538 -.0837	-0.1781 .1953 .2358 -.0837	0.1399 4440 1.490809 1776	1.9453 -1.9937 0803 -9620	(1)
G.....			1.0000 .901482 8400 -1.0000	0.4270 -1475 -1909 2700	0.1456 -1475 -1909 2700	-0.0906 -.1767 -.0565 -.0798	0.6848 .3741 .013538 -.0837	-0.1781 .1953 .2358 -.0837	0.1399 4440 1.490809 1776	1.9453 -1.9937 0803 -9620	-2
F.....			1.0000 .787767 3433 -1.0000	0.4270 -1475 -1909 2700	0.1456 -1475 -1909 2700	-0.0906 -.1767 -.0565 -.0798	0.6848 .3741 .013538 -.0837	-0.1781 .1953 .2358 -.0837	0.1399 4440 1.490809 1776	1.9453 -1.9937 0803 -9620	-2
E.....			1.0000 .947750 .5086 -1.0000	0.4270 -1475 -1909 2700	0.1456 -1475 -1909 2700	-0.0906 -.1767 -.0565 -.0798	0.6848 .3741 .013538 -.0837	-0.1781 .1953 .2358 -.0837	0.1399 4440 1.490809 1776	1.9453 -1.9937 0803 -9620	-1
D.....			1.0000 .660942 .5648 -1.0000	0.4270 -1475 -1909 2700	0.1456 -1475 -1909 2700	-0.0906 -.1767 -.0565 -.0798	0.6848 .3741 .013538 -.0837	-0.1781 .1953 .2358 -.0837	0.1399 4440 1.490809 1776	1.9453 -1.9937 0803 -9620	-5
Beta.....	CA (+ .1270	CB + .3273	CI + .0142	CH + 1262	CG - 0539	CF - 2249	CE + 1675	CD + .4073			

1 Checked to fourth place exactly.

$$R = \sqrt{.64707443} = 0.8044$$

TABLE 8.—The necessary numbers and products, in addition to those found in tables 1 and 2, to complete the calculation of the "B" multiple correlation coefficient where nine characters are involved

	A	I	H	G	F	E	D	C	B	S	Accuracy
I.....		1.0000 9759 -1.0000	0.4270 0473 -0171	0.01456 0949 -0024	-0.00095 -1619 -1689	0.6848 .6501 -1773	-0.1781 -1773 -1776	0.1399 -1307 -1309	-0.00980 -0.0118 -0.0121	1.9168 -1.9641 -1.9641	-2
H.....			1.0000 983998 8140 -1.0000	-1475 -14704 -2284 -2781	-1787 -234763 -1872 -2054	3711 345815 -0745 -0915	199171 190171 -2715 -3335	-4446 -437136 -3826 -4700	3251 3220 3269 -4016	2.276021 2.276021 1.4759 -1.8132	+1
G.....				1.0000 913505 8403 -1.0000	7324 -597408 5675 -6753	-0836 -002161 -0490 -0571	1574 -148401 -0552 -0656	-2948 -302152 -2901 -2489	-1025 -1096 -0175 -0208	863747 1.0779 -1.2828 -1.2828	-1
F.....					1.0000 788319 3449 -1.0000	-0457 -148332 -0072 -0210	1735 -159455 -0652 -2759	-3173 -344381 -1029 -2984	-0282 -0394 -0376 -1090	298513 -1918 -5560 -5560	+3
E.....						1.0000 950003 5072 -1.0000	1903 -183458 -0940 -1858	1994 186208 0543 -1072	0528 0474 0236 -0465	1.845493 -9681 -9681	-3
D.....							1.0000 999064 8304 -1.0000	6294 631205 4948 -5958	5754 5761 4786 -5763	1.740926 1.8037 -2.1721 -5763	(c)
C.....								1.0000 996519 4158 -1.0000	6250 6250 1921 -4621	2.360233 6081 -1.4626 -1.4626	+5
Beta.....	BA -1343	BI -0394	BH +1175	BG -1051	BF +3288	BE +0527	BD +3010	BC +4621			

$$R = \sqrt{0.50218995} = 0.7087$$

¹ Checked to fourth place exactly.

TABLE 9.—The necessary numbers and products of the calculations to the completion of the "A" multiple correlation coefficient where nine characters are involved

	I	II	G	F	E	D	C	B	A	S	Accuracy
I...		{ 0 4270 - 4270 1.0000 8177 -1.0000	0 1456 - 1456 - 1475 - 2097 2564	-0.0906 - 0906 - 1787 - 1380 - 1688	0.0848 - 0848 .3741 .0817 - 0969	-0.1781 - 1781 .1953 .2713 - 3318	0 1399 - 1399 4446 3849 - 4707	-0.0080 - 0080 .3251 .3285 - 4019	0 1554 - 1554 .1265 0601 - 0736	2.2760 - 2 5684 1 5965 1 9525	-1
H...			{ 1 0000 .9250 -1.0000	.7324 .7102 - 7678	.0636 - 0152 .0164	- 1574 - 0619 0609	- 2848 - 2065 .2232	- 1027 - 0171 .0185	2941 2866 - 3101	1 5435 1 6215 1 7529	(1)
G...				{ 1.0000 .4232 -1.0000	- 0457 - 0418 - 0688	- 1735 - 0903 1921	- 3173 - 0813 - 0637	- 0282 .0397 - 6213	4590 2629 - 6213	1 3594 5901 - 1 3944	+3
F...					{ 1.0000 5185 -1.0000	- 1903 - 0869 1677	1994 0698 - 1346	.0528 0213 - 0410	.2236 0885 - 1734	2 3623 6125 - 1 1813	(1)
E...						{ 1.0000 .8376 -1.0000	6294 5060 - 6041	5754 .4764 - 5688	- 0306 .0712 - 0851	1 6702 1 8913 - 2 2580	(1)
D...							{ 1.0000 .4227 -1.0000	6264 1866 - 4403	0590 0684 - 1619	2 4066 6770 - 1.6024	+2
C...								{ 1.000 5102 -1.0000	.0243 .0922 1808	2.4653 4178 - 8188	4
B...											
Beta..	{ A1 + 0721	+ AH + 0779	+ AG - 1552	+ AF + 6788	+ AE + 1554	+ AD + .0120	+ AC + 2415	+ AB + 2415	- AB - 1808	-	-

1 Checked to fourth place exactly

$$R = \sqrt{0.33030087} = 0.5745$$

THE CALCULATION

Following Wallace and Snedecor's method of solving normal equations, the headings of the columns, table 1, are from A to I and the sum column. To the left are the blocks from A to H, inclusive. Each block has 4 lines, except the A block, which needs only 2. Of the 4 lines, only 3 have any use in the first table and the last (table 9) in the calculation of a full set of multiple coefficients. In line 1 are entered the simple correlation coefficients which have previously been calculated. Line 2 of the first block (block A in table 1) carries the simple coefficients of that block, with the sign changed. Line 3 of the other blocks carries the sum of the columns where it occurs, and line 4 the quotients of the sums in line 3 divided by the first sum in each block. These are the only figures necessary to enter in the table, as they are the only ones used to calculate the rest of the table below, the partial regression coefficients (the betas), and the multiple correlation coefficients.

In obtaining line 3 of the second block (B of table 1) only one multiplication is to be made in each column; in the C block, two multiplications; and for each succeeding block one more multiplication. Taking block B and column B, for example, put 1.0000 on the calculator on the left column of the keyboard. Shift dials to the right and enter the 1.0000 in the lower dial. Then mark the decimal place on the lower dial with a marker, and this place will always be the same throughout all calculations in the multiple correlation tables. At the beginning of each new operation the decimal points of the keyboard and the lower dial must be alined before the number is placed on the machine. On the writer's machine, a 1929 8-bank model, the decimal is between the fourteenth and fifteenth places on the lower dial.

After the 1.0 is entered on the machine in the proper place, one of the multipliers is entered on the keyboard. In table 1, this multiplier (line 1, block A, column B) is 0.0243, and is entered on the keyboard where the decimal is one place farther to the right than the decimal on the lower dial. This arrangement allows the multiplication to go forward, and by using the first place, left to right, on the upper left dials, there will be eight places covered on the lower dial to the right of the decimal point when both multipliers have four decimal places. Of course, the decimal place in the upper dials will be to the left of the first place. However, this multiplier can be placed on the keyboard with the decimal place the same as was the coefficient, but before multiplying the carriage must be shifted to the left one place.

Now, after 0.0243 is entered on the keyboard the multiplication with -0.0243 (block A, column B, line 2) can be made by using the subtraction switch. (The -0.0243 will be one of the multipliers for all multiplications in the calculation of the B block.) In other words, the multiplying can be done by subtracting from 1.0000. The result is 0.9994, which is entered in line 3, column B, block B, and is the number to be used to divide into all other figures of line 3 of B block to get line 4. Now, clear the machine of all figures in dials and keyboard. Enter in the lower dials the BC coefficient of line 1, column C, block B, which is 0.6264. Clear the keyboard and enter on it 0.0590 line 1, column C, block A, and multiply by subtracting -0.0243 , which leaves 0.6250. This is entered on the table in line 3, column C. Clear the top dial and keyboard only and immediately divide by 0.9994 and enter the quotient, 0.6253, in line 4, column C and block B. Clear the machine and enter on the lower dial the BD coefficient, 0.5754; looking just above the BD coefficient in line 1, block A, take -0.0306 and enter it on the keyboard and multiply it by -0.0243 by using the plus switch. Adding the product to 0.5754 gives 0.5761, which goes in line 3, column D, block B. This figure is on the machine ready to be divided by 0.994 when the top dials and keyboard are cleared. The latter calculation gives 0.5765 and is entered in line 4, column D, block B, with its sign changed. This process goes on, taking up each coefficient of block B and subtracting from or adding to the product of the figure in line 1 of block A, of the same column as the coefficient, with the figure in line 2 of column B of block A. The sum is taken in the same way as the coefficients. The check figure, of course, is always line 4, column S, of each block and should equal everything to its left in the same line.

In block C there will be two multiplications added to or subtracted from the coefficients of line 1. Take column C, block C, for instance. Enter 1.0000 on the machine as before; in block A, column C, there is found 0.0590 and -0.0590 to be multiplied together and subtracted at the same time from 1.0000. Then in block B, lines 3 and 4 of column C, there are 0.6250 and -0.6253 , which will be handled in a similar manner after clearing the top dials and keyboard. This leaves, after these two multiplications, 0.6057, which when divided by itself gives -1.0000 . This goes in line 4 with 0.6057 just above in line 3. The next procedure is to clear the machine of all numbers. Then enter 0.6294, the coefficient CD, in the proper place on the lower dial and multiply by addition -0.0306 , line 1, block A, of the same

column as the coefficient CD, by -0.0590 , line 2, column C, block A. Line 4 in each block just above the first column of the block being calculated is always one of the multipliers throughout that block. The other multiplier is in each block above the coefficient, but in line 3. Continuing with column D, block C, after the A block multiplication has been made and added to the 0.6294 , the next multiplication will be found in the next lower block as denoted above; which is 0.5761 and -0.6253 ; leaving when subtraction by multiplication is done, 0.2710 . Then dividing by 0.6057 without clearing the lower dial gives -0.4474 when the sign is changed.

One more illustration will probably clarify all the details of the calculation. Passing to block H, first take column H and put 1.0000 on the lower dial; then make the following multiplications, starting by subtracting each from the number on the lower dial: (0.1265×-0.1265) of block A; (0.3220×-0.3222) of block B; (0.2358×-0.3893) of block C; (-0.0920×0.1685) of block D; (0.2222×-0.2914) of block E; (-0.0789×0.1285) of block F; and (-0.0155×0.0355) of block G. The remainder is 0.6975 , which is entered in line 3, column H, block H, with -1.0000 just below in line 4.

Then, taking the next coefficient in block H, which is HI or 0.4270 , and entering it on the cleared machine, the following multiplications are made, off or on according to the sign: (0.1554×-0.1265) of block A; (-0.0118×-0.3222) of block B; (0.1381×-0.3893) of block C; (-0.2283×0.1685) of block D; (0.4974×-0.2914) of block E; (-0.0474×0.1285) of block F; and (0.1488×0.0355) of block G. The remainder, 0.1732 , is entered in line 3, column I, block H. While still on the machine it is divided by the first number in line 3 of block H, which is 0.6975 . This leaves -0.2483 when the sign is changed.

There is one point that may confuse some operators of calculating machines. It frequently happens in subtracting and multiplying at one operation, that the product of the multiplication is larger than the number from which it is being subtracted. When the bell rings, indicating that the former is larger than the latter, proceed with the multiplication until it is completed. When it is finished the complement of the number than on the machine can easily be obtained, and by adding it to the number and clearing the machine the complement is checked. When the complement clears the machine it can be entered immediately on the machine, and the usual division can be made. Should the number that is being subtracted from be exhausted before all multiplications are completed, the other multiplications can be made before it is necessary to know the result.

THE GENERAL SET-UP

If all possible multiple correlation coefficients are desired, skeleton tables for all variables should first be made out. The skeleton tables should then be filled in with the simple correlation coefficients and the sum of each block entered in the proper place. In the calculation of the first table there are certain numbers that can be used in some of the other tables. After completing the first two blocks there is a place in one of the tables for each multiplication when it is completed. This is the reason for the second line in each block. When calculation is being performed in tables where line 2 is filled in it is only necessary to pick up the number in line 2 and complete the multipli-

cations in the table above the block in question to finish that particular column and block. To illustrate the use of line 2, take block C, column C, of table 1; enter 1.0000 in the lower dial, multiply and subtract 0.0590×-0.0590 , and record the remainder (0.996519) in line 2, column C, block C, of table 8. To illustrate this further, take in table 1, column H, block G, the coefficient in line 1, -0.1475 , and enter it on the lower dial. Then the remainder (-0.184704) of the first multiplication (0.1265×-0.2941) by addition (the coefficient in negative) is placed in table 8, line 2, column G, block H. Now, clear the top dials and keyboard and make the B block multiplication (0.3220×0.1097) (by subtraction). The remainder (-0.149380) (on the lower dial) is placed in table 7, column G, block H, line 2. The multiplication in block C (table 1) is now made, which remainder (-0.058432) is entered in table 6, column G, block H. The block D multiplication is then completed and the remainder (-0.055175) recorded in table 5, column G, block H. Then the remainder of block E multiplication goes in table 4, column G, block H; and the block F multiplication remainder, of course, is recorded in line 3, column H, block G, of table 1.

In any of the calculations of the first table involving column I (the primary of first table) or block I of table 2, the remainders are recorded in line 3 of the tables, blocks, and column where they belong. In the tables other than tables 1, 2, and 9, all sums of block I which are so recorded in line 3 are complete and ready for division by the division number of that block without further multiplication. A glance at the tables will make this evident. Line 3 in the I blocks and line 2 of all other blocks of the last column (not sum column) of each table, other than tables 1 and 9 and columns I and S, block I of table 2, can be obtained by taking them direct from table 1. For instance,

0.2283 in table 6, column D, block I, occurs in line 3, column I, of block D in table 1. Also, passing down column D of table 6 on line 2, read -0.0920 , 0.0193 , 0.0063 , -0.2808 . Then look in table 1, block D, and read from right to left on line 3 the same figures in their respective columns.

CALCULATION OF TABLES OTHER THAN 1 AND 9

It must be remembered that the multiple coefficient of the last table of a series of multiple coefficients (table 9 here) must be calculated as a whole and separately from the other tables. In other words, there are no calculations in the other tables that can be used in its calculation. As previously stated, certain calculations from table 1 can be used for the other tables (tables 2 to 8 herein). These remainders of multiplications subtracted from or added to a coefficient were placed in line 2 of the corresponding block and columns where they belong. The exception to this, as has been noted, occurs in all the "I" blocks, where such sums were placed in line 3 of their columns.

To illustrate the calculation of the tables other than 1 and 9, table 4 will be used. Line 3 of block I and line 2 of blocks H and G have been filled in from calculation of table 1, with the exception of column F. In column F, line 3 of block I and line 2 of blocks H and G can be written in from block F of table 1; -0.0474 column I, -0.0789 column H, and 0.4754 column G. Then, divide line 3, block I, through by 0.5243 , obtaining line 4. Next, block H: Enter the sum of HH of

line 2 (0.708201) on the keyboard, align the decimal points, transfer to the lower dials, enter 0.1740 (line 3 IH) on the keyboard one place to the right of the coefficient decimal place (or shift carriage one place to the left), and multiply by -0.3319 by subtraction. The result is 0.6505, which is entered in line 3, column H, block H; enter -1.000 just beneath.

The other columns and blocks are calculated similarly. The only difference is the entering on the machine of the number in line 2 instead of the coefficient in line 1; and with the completion of the calculations of each table as outlined, the figures necessary to calculate the partial regression coefficients and the multiple coefficient are obtained.

CALCULATION OF THE PARTIAL REGRESSION COEFFICIENTS

In the calculation of the partial regression coefficients or the betas it is seen that these betas are placed in certain columns. In table 1, I being used as the primary, the "I" betas are found. Beta IH is in column H, IG in column G, etc. In the other tables the betas appear in their corresponding columns. Table 1 may be used to illustrate the method of calculating the betas. One beta (IH) comes out at the completion of the main body of the table as the first unknown that is found. The only change is to reverse the sign. Therefore, beta IH, $+0.2483$, is entered in the beta line in column H. To get beta IG, enter on the calculator (with reverse sign) the number found in line 4, column I, block G; which in this case is 0.3416. Then multiply the figure 0.0355 in block G, column H, line 4, with beta IH, 0.2483, by addition or by using the plus switch. The beta IG is then read direct from the calculator. To arrive at beta IF, use line 4 in block F. Starting with column I, enter on the machine -0.0771 and multiply 0.1285×0.2483 by subtraction; then -0.7741×0.3504 by addition and read beta IF direct from the machine. Beta IF is then entered in column F. The other betas are calculated in a similar manner, using the numbers in line 4 in each block corresponding to the beta desired and entering first the number in column I (the primary column) on the machine with reversed sign and multiplying by subtraction or addition the figures in each column of line 4 with the corresponding beta previously calculated.

In calculating betas in the tables other than 1 and 9, it is necessary to use certain parts of table 1. For illustration, take table 6; beta DI is calculated from the I block of table 6, but to get DC it is necessary to go to block C of table 1. Then, entering -0.4474 , which appears in column D, line 4, on the calculator with the reverse sign, take the other line 4 numbers in block C and multiply them, by subtraction or addition as their sign dictates, by their corresponding betas; the number in column E by beta DE; column F, by beta DF; column G, by beta DG; column H, by beta DH; and column I by beta DI. The beta DC is then read directly from the machine. All the other partial regression coefficients can be obtained in a similar manner.

The accuracy of the calculations of each block is indicated in the last column of each table as the difference in fourth decimal place to check figure.

The other operations, such as checking the betas by means of substituting in one of the equations and the calculation of R , are the same as given in Wallace and Snedecor (2).

The time required to calculate a set of nine multiple tables like tables 1 to 9 is about 10 hours. This does not take into account certain errors that have to be corrected that may greatly delay the completion of the calculation.

SUMMARY

Certain methods of machine calculation of multiple correlation coefficients are reported.

In the multiplication by subtracting from or adding to another number and carrying the totals on the machine until time to divide, and at that time having the number in a position where it can be divided without putting it in or taking it out of the machine, makes possible the short cut in methods described here.

These short methods of calculation reduce the time necessary to obtain the multiple correlation coefficients and give a higher degree of accuracy to the work.

SEROLOGICAL STUDIES OF SWINE ERYSIPELAS WITH PARTICULAR REFERENCE TO AGGLUTINATION¹

By H. W. SCHOENING, *chief*, and G. T. CREECH, *veterinarian, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

When the results of previous investigations² on the agglutination test for swine erysipelas were reported, information was not available as to the time of the appearance and stability of agglutinins in the blood stream of swine following infection. To obtain data on this point, a series of observations and agglutination tests on artificially infected swine was made. Information on the subject also has recently been received from the field. These data are reported in the present paper, together with other experimental data obtained from the agglutination tests of blood serum from swine and from persons who were suspected of being infected with *Erysipelothrix rhusiopathiae*. A rapid serological method of identifying cultures of *E. rhusiopathiae* is also reported.

TIME OF APPEARANCE AND STABILITY OF AGGLUTININS IN THE BLOOD OF ARTIFICIALLY INFECTED PIGS

EXPERIMENTAL PROCEDURE

Eight pigs were used in the experiment made to determine the time of appearance and stability of agglutinins in the blood stream of artificially infected swine. Two of the pigs were 3 weeks of age, and the remainder were from 3 to 4 months of age.

All the pigs were exposed to swine-erysipelas infection by injection of 20 cc of broth cultures of *Erysipelothrix rhusiopathiae*, either intravenously, subcutaneously, or intraperitoneally, or by combinations of these methods. Before exposure, samples of serum were drawn and the rapid whole-blood and serum agglutination tests were made for swine erysipelas. All these tests were negative.

Beginning about 3 days after the exposure of the pigs to infection blood was drawn and samples of serum were collected daily for several days, carbolyzed 0.5 percent, and stored in the ice box for use in the tube test. The rapid plate test was made with the whole blood and serum at the time of collection of the samples. Later, samples were collected at 2- or 3-day intervals, in most instances, and tests were made as stated above. Toward the end of the experiment, bleedings were made at lengthening intervals of from 1 to 3 weeks. All the serum samples collected on the various dates were then tested against the same antigen by means of the tube test, the technic of which has already been described.³

¹ Received for publication Aug. 22, 1934; issued April, 1934.

² SCHOENING, H. W., and CREECH, G. T. AN AGGLUTINATION TEST FOR SWINE ERYSIPELAS. *Jour. Amer. Vet. Med. Assoc.* (n. s. 35) 82: 503-509. 1933.

—CREECH, G. T., and GREY, C. G. A LABORATORY TUBE TEST AND A WHOLE-BLOOD RAPID AGGLUTINATION TEST FOR THE DIAGNOSIS OF SWINE ERYSIPELAS. *North Amer. Vet.* 13 (12): 19-25. 1932.

³ SCHOENING, H. W., CREECH, G. T., and GREY, C. G. See footnote 2.

RESULTS

Six of the pigs became noticeably ill following exposure, but all recovered. Two of the pigs, nos. 4259 and 4260, 3 weeks of age, failed to show any clinical evidence of infection after exposure, and tests of their blood remained consistently negative.

The results of the various tests on each of the six pigs which showed clinical evidence of infection are recorded in table 1. With the exception of the preinfection bleedings, the table records only the results of those tests made after the first indications of reactions were noted.

TABLE 1.—Results of agglutination tests¹ for swine erysipelas of individual pigs inoculated by various methods with 20 cc of a bouillon culture of *Erysipelothrix rhusiopathiae*

PIG 4253, INOCULATED FEB 25, 1933, INTRAVENOUSLY, SUBCUTANEOUSLY, AND INTRAPERITONEALLY

Date of drawing blood	Results of plate test ² of		Results of tube test ³ of blood serum at dilutions of —							Remarks
	Whole blood	Blood serum	1. 25	1. 50	1. 100	1. 200	1. 500	1, 000		
Feb. 25	—	—	—	—	—	—	—	—	Blood drawn prior to inoculation. Temperature of 104.5° F., slight depression, off feed. Temperature of 104.7°, recovery of appetite, no symptoms. Temperature of 104.2°, no symptoms. Temperature of 104°, no symptoms. Temperature of 102°, serum sample decomposed. Temperature normal on this and subsequent dates.	
Mar. 3..	(?)	+	+	+	+	+	—	—		
Mar. 6 ...	+	+	+	+	+	+	—	—		
Mar. 9 ...	+	+	+	+	+	+	—	—		
Mar. 13 ...	+	+	+	+	+	—	—	—		
Mar. 22 ...	+	+	+	P	S	—	—	—		
Mar. 29...	+	+	+	+	+	—	—	—		
Apr. 11...	—	—	P	VS	—	—	—	—		
May 9	—	—	P	VS	—	—	—	—		
May 25 ..	—	—	+	P	—	—	—	—		
June 11 ..	—	—	+	P	—	—	—	—		

PIG 4254, INOCULATED FEB 25, 1933, INTRAVENOUSLY

Feb 25	—	—	—	—	—	—	—	—	Blood drawn prior to inoculation. Temperature of 106.5° F., marked depression, off feed. Temperature of 104.5°, recovery of appetite, evidence of arthritis in all 4 feet. Temperature of 105.4°, well-marked arthritis. Temperature of 104.6°, swelling of joints still noticeable. Temperature of 104.1°, joints appeared normal. Normal after this date, serum sample decomposed.
Mar 3	+	+	+	+	+	+	—	—	
Mar 6	+	+	+	+	+	+	S	—	
Mar 9	+	+	+	+	+	+	—	—	
Mar 13	+	+	+	+	+	—	—	—	
Mar 22	+	+	+	+	—	—	—	—	
Mar 29	+	+	P	S	—	—	—	—	
Apr 11	+	+	—	S	—	—	—	—	
Apr 25	+	+	+	+	—	—	—	—	
May 9	+	+	+	P	—	—	—	—	
May 25	+	+	P	S	—	—	—	—	
June 16	+	+	P	S	—	—	—	—	

¹ Key: + = complete agglutination, P = partial agglutination; S = slight agglutination; VS = very slight agglutination; — = no agglutination, (?) = result questionable.

² Plate tests were made on same date blood was drawn.

³ All tube tests were made on June 21 with the same lot of antigen.

TABLE 1.—Results of agglutination tests for swine erysipelas of individual pigs inoculated by various methods with 20 cc of a bouillon culture of *Erysipelothrix rhusiopathiae*—Continued

FIG 4255, INOCULATED MAR 31, 1933, INTRAVENOUSLY AND INTRAPERITONEALLY

Date of drawing blood	Results of plate test of--		Results of tube test of blood serum at dilutions of--						Remarks
	Whole blood	Blood serum	1:25	1:50	1:100	1:200	1:500	1:1,000	
Mar. 31.	—	—	—	—	—	—	—	—	Blood drawn prior to inoculation. Temperature of 105.4° F., off feed, depression
Apr. 3	—	+	—	—	—	—	—	—	
Apr. 4	—	+	+	+	+	VS	—	—	Temperature of 105.4°, depression Temperature of 105°, appeared brighter, ate fairly well
Apr. 5	+	+	+	+	+	S	—	—	
Apr. 6	+	+	+	+	+	+	—	—	Temperature of 105.4°, appeared brighter, eating.
Apr. 7	+	+	+	+	+	+	—	—	
Apr. 10	+	+	P	+	+	+	—	—	Temperature of 104.6°.
Apr. 17	+	+	+	+	+	P	—	—	
Apr. 24	+	+	+	+	+	P	—	—	Temperature of 103.6° Normal after this date.
May 9	+	+	+	+	+	VS	—	—	
May 25	+	+	+	P	—	—	—	—	
June 16	?	+	+	S	—	—	—	—	

FIG 4256, INOCULATED MAR 31, 1933, INTRAVENOUSLY AND INTRAPERITONEALLY

Mar. 31	—	—	—	—	—	—	—	—	Blood drawn prior to inoculation Temperature of 106.6° F., depression, off feed
Apr. 3	—	—	+	VS	—	—	—	—	
Apr. 4	—	+	+	+	—	—	—	—	Temperature of 106.2°, depression, lame right front leg Temperature of 105.8°, ate fairly well, lame in all 4 feet
Apr. 5	+	+	+	+	+	+	—	—	
Apr. 6	+	+	+	+	+	+	P	—	Temperature of 106°, still lame Temperature of 105.8°, lameness not so pronounced
Apr. 7	+	+	+	+	+	+	S	—	
Apr. 10	+	+	+	+	+	+	+	+	Temperature of 104°, only slight lameness Normal after this date.
Apr. 17	+	+	+	+	+	+	S	—	
Apr. 24	+	+	+	+	+	P	S	—	
May 9	+	+	+	+	+	S	—	—	
May 25	+	+	+	+	+	S	—	—	
June 16	?	+	+	+	P	S	—	—	

FIG 4257, INOCULATED APR 27, 1933, INTRAVENOUSLY

Apr. 27	—	—	—	—	—	—	—	—	Blood drawn prior to inoculation. Temperature of 106.2° F., depression, off feed.
May 1	—	+	+	P	?	—	—	—	
May 3	+	+	+	+	+	+	—	—	Temperature of 105°, off feed. Temperature of 104.8°, lame in all 4 feet, joints hot, swollen, and painful.
May 5	+	+	+	+	+	+	P	—	
May 6	+	+	+	+	+	+	S	—	Temperature of 105°, lame in all 4 feet, joints hot, swollen, and painful
May 15	+	+	+	+	+	S	—	—	
May 25	+	+	+	+	+	S	—	—	Temperature normal, little evidence of lameness Knee and hock joints slightly enlarged. Do.
June 16	+	+	+	+	+	S	—	—	

FIG 4258, INOCULATED APR. 27, 1933, INTRAVENOUSLY

Apr. 27	—	—	—	—	—	—	—	—	Blood drawn prior to inoculation. Temperature of 106° F., no visible symptoms.
May 1	—	—	+	S	—	—	—	—	
May 3	+	+	+	+	+	+	—	—	Temperature of 106.5°, depression, off feed.
May 5	+	+	+	+	+	+	—	—	
May 6	+	+	+	+	+	P	—	—	Temperature of 103.5°, ate fairly well, slightly lame. Temperature of 103.5°, lame, swelling of knee and hock joints.
May 15	+	+	+	+	P	—	—	—	
May 25	+	+	+	+	P	—	—	—	Normal after this date.
June 16	(?)	+	+	+	P	—	—	—	

DISCUSSION

The first symptoms of the disease in the pigs were observed from the second to the third day after inoculation and were usually well marked on the fourth day. Agglutinins were first detected in the blood of the infected pigs from the third to the sixth day after inoculation.

Pigs 4255 and 4256 gave positive reactions to both the whole-blood plate test and the tube test 2 days after the first symptoms were observed, whereas the other 4 infected pigs did not give definite positive reactions to both tests until the third day after the first evidence of the disease.

These observations indicate that agglutinins make their appearance early in the disease, within a few days after the animal shows some clinical evidence of infection. Field observations by C. F. Harrington,⁴ a veterinary inspector of the Bureau of Animal Industry, in outbreaks of swine erysipelas in South Dakota, confirm these findings. In a number of cases he applied the whole-blood plate test to animals sick but a short time and obtained positive results in some animals that were sick for only 2 or 3 days.

It is of interest to note the rapidity with which the agglutinins are formed in the blood after a certain stage of the disease. For example, pigs 4253 and 4254 gave entirely negative results to the plate and tube tests for swine erysipelas on the fifth day after inoculation, but 24 hours later they gave strong reactions to the plate test and definite positive reactions to the tube test also.

Table 1 shows the high points of the reactions to the tube tests in the different experimental pigs, the highest reactions usually occurring about the second week after infection had taken place. There was a gradual loss of reactions after the second week.

The results seem to indicate that pigs suffering only slight attacks of the disease, or those which make a prompt recovery, probably lose their reactions more or less after a comparatively short time. This finding is in keeping with field observations in which animals that had recovered from the disease without showing any marks of it gave negative reactions to the test, whereas animals in the same herd with arthritis gave positive reactions. Available data on serological tests of field cases show that pigs which have developed chronic lesions, particularly cases of chronic arthritis, will continue to give well-marked reactions for an indefinite period.

AGGLUTINATION TESTS OF SWINE WITH THE URTICARIAL FORM OF SWINE ERYSIPELAS (DIAMOND SKIN DISEASE)

In 1933 a sample of blood serum and a specimen of skin, from the same pig, showing typical diamond skin lesions were received from G. W. Stiles, Jr., in charge of the branch Pathological Laboratory of the United States Department of Agriculture at Denver, Colo. *Erysipelothrix rhusiopathiae* was recovered from the specimen of skin, but the serum sample gave negative results to both the plate and tube agglutination tests for swine erysipelas.

Stiles⁵ has reported five cases of typical diamond skin disease in swine, detected on post-mortem examination at federally inspected

⁴ Personal communication.

⁵ STILES, G. W., and DAVIS, C. L. SWINE ERYSIPELAS AND ITS ECONOMIC IMPORTANCE. Jour. Amer. Vet. Med. Assoc. (n. s. 37) 84: 895-906, illus. 1934.

establishments, in which negative reactions to the plate agglutination test were obtained. In these cases, however, bacteriological examination of the affected skin or other tissues or both failed to disclose *Erysipelothrix rhusiopathiae*. The reason for the negative serological results in these cases of skin lesions has not been determined.

PASSIVE TRANSFER OF AGGLUTININS TO SWINE INJECTED WITH ANTI-SWINE-ERYSIPELAS SERUM

In an investigation of an outbreak of disease in a swine herd in South Dakota, C. H. Hays, inspector in charge of the Federal field station at Pierre, S. Dak., obtained a strong positive reaction to the whole-blood plate agglutination test for swine erysipelas in the case of a sick hog which died the following day. The clinical observations, post-mortem examination, and laboratory studies revealed the disease in this herd to be a suispestifer infection with no evidence of erysipelas infection whatsoever. However, this hog was in a herd which was used for studying the efficacy of anti-swine-erysipelas serum in the field, and the animal, when taken sick, had been given two injections of 25 cc each of the serum about a week previous to the test. Since it had been determined that there was no erysipelas infection in this hog, Hays raised the question whether the positive reaction in the animal was due to the injection of the anti-swine-erysipelas serum. To obtain information on this point, pig 1, weighing 20 pounds, was injected subcutaneously with 30 cc of anti-swine-erysipelas serum of equine origin. Pig 2, about the same age, size, and weight as pig 1, was given an injection of 30 cc of normal horse serum subcutaneously. Table 2 gives the results of the plate and tube agglutination tests on samples of serum drawn on different days from these animals.

TABLE 2. Results of agglutination tests¹ for swine erysipelas of pigs injected subcutaneously with anti-swine-erysipelas serum (horse) or normal horse serum

FIG 1, INJECTED OCT. 5, 1933, WITH 30 CC ANTI-SWINE-ERYSIPELAS SERUM (HORSE)

Date of drawing blood	Results of plate test ² of --		Results of tube test ³ of blood serum at dilutions of --						
	Whole blood	Blood serum	1:25	1:50	1:100	1:200	1:500	1:1,000	1:2,000
Oct. 5 ⁴	—	—	—	—	—	—	—	—	—
Oct. 6	+	+	+	+	+	+	+	+	—
Oct. 7	+	+	+	+	+	+	+	+	—
Oct. 9	+	+	+	+	+	+	+	+	—
Oct. 11	+	+	+	+	+	+	+	—	—
Oct. 17	+	+	+	+	+	+	P	—	—
Oct. 28	+	+	+	+	+	+	VS	—	—
Nov. 6	+	+	+	+	+	—	—	—	—

FIG 2, INJECTED OCT. 9, 1933, WITH 30 CC NORMAL HORSE SERUM

Oct. 9 ⁴	—	—	VS	—	—	—	—	—	—
Oct. 10	—	—	P	—	—	—	—	—	—
Oct. 11	—	—	S	—	—	—	—	—	—

¹ Key: + = complete agglutination; P = partial agglutination; S = slight agglutination; VS = very slight agglutination; — = no agglutination

² Plate tests were made on same date blood was drawn.

³ All tube tests were made on Nov. 7 with the same lot of antigen.

⁴ Sample was taken before injection of serum.

The injection of the anti-swine-erysipelas serum into pig 1 produced agglutinins detectable 24 hours later by both the rapid whole-blood plate test and the tube test. The serum reacted to the tube test in a dilution of 1 to 1,000, 24 hours after the injection. The titer gradually decreased from this point, but agglutinations in a dilution of 1 to 100 were still in evidence after 1 month. The whole-blood plate test was still positive at this time, but the reaction was not so pronounced and did not develop so rapidly, although it was well marked, within 2 minutes. The injection of the normal horse serum into pig 2 failed to cause any agglutinin response.

In tests of the agglutination titer of the anti-swine-erysipelas serum (horse) injected into pig 1, positive reactions were obtained at dilutions of from 1 to 25 to 1 to 5,000. The serum was negative, however, in a dilution of 1 to 10,000. In similar tests of the normal horse serum used in the injection of pig 2, a dilution of 1 to 25 resulted in a positive reaction, all higher dilutions being negative.

To calculate data on the transfer of the specific agglutinins from the serum of one animal to that of another, the following procedure was carried out, with the results given.

Pig 2, which was injected with normal horse serum and which, as previously stated, was of about the same age and weight as pig 1, was bled out as completely as possible, to determine the approximate quantity of blood in pig 1 and the approximate quantity of serum that could be obtained from that animal. The total quantity of blood collected was 440 cc, from which 200 cc of serum was obtained.

Pig 1 had been injected subcutaneously with 30 cc of anti-swine-erysipelas serum with a titer of between 1 to 5,000 and 1 to 10,000. This specific serum could thus be considered to have been diluted about seven times. After the injection of the specific serum, the titer of the pig serum, which was between 1 to 1,000 and 1 to 2,000, would indicate that certainly a large portion of the agglutinins contained in the specific serum had been transferred to the blood of the normal pig injected.

AGGLUTINATION TESTS WITH HUMAN SERUM

In the course of experimental work on the virulence of *Erysipelothrix rhusiopathiae* for rabbits and other animals, a research worker in one of the State institutions developed an infection on the hand following direct contact of the hand with a skinned carcass of a rabbit dead after inoculation with *E. rhusiopathiae*. The first symptoms were noted July 30, 1932, in a finger 3 days after contact with the rabbit carcass. There was swelling, which later extended to other digits as well as to the hand itself, with a discoloration of the skin. The member became painful and there was a general systemic reaction. The condition persisted for some months and finally cleared up under local antiseptic treatment.

There had been no opportunity to attempt to recover *Erysipelothrix rhusiopathiae* from the patient. The diagnosis of erysipelas infection rested on the clinical symptoms and history of the case. However, samples of blood serum were collected August 31, September 24, and December 16, 1932, and the agglutination test for swine erysipelas was made. The results of the test are given in table 3.

TABLE 3.—Results of agglutination tests ¹ for swine erysipelas on samples of human blood serum

[Date of exposure, July 27, 1932]

Date of collecting sample	Results of plate test of blood serum	Results of tube test of blood serum at dilutions of—						
		1:25	1:50	1:100	1:200	1:500	1:1,000	1:2,000
Aug. 31.	+	+	+	+	+	+	+	—
Sept. 24.	+	+	+	+	+	+	+	—
Dec. 16.	—	—	—	—	—	—	—	—

¹ + = complete agglutination; — = no agglutination

To check the specificity of these reactions, 10 samples of serum from apparently normal individuals were tested by both the plate and tube agglutination at the same time and against the same antigen. All 10 samples gave negative results to the plate test, and 7 were entirely negative to the tube test. Three of the samples gave some evidence of agglutination in the tube test at dilutions of 1 to 25 and 1 to 50.

Although no bacteriological confirmation of the diagnosis of infection with *Erysipelothrix rhusiopathiae* in this case was obtained, the history, clinical symptoms, and results of the serological tests leave little doubt as to the correctness of the diagnosis. The agglutination reaction parallels rather closely the results obtained in certain types of infection in swine.

AGGLUTINATION PLATE TEST WITH WHOLE BLOOD OF MAN HAVING CHRONIC ARTHRITIS

Since chronic arthritis is frequently a sequel of infection with *Erysipelothrix rhusiopathiae* in swine and the serums of animals so affected give strong positive reactions to the agglutination test for swine erysipelas for considerable periods, and since man is known to be susceptible to infection with this organism, a rapid, whole-blood agglutination test was made on the blood of a veterinarian with a chronic polyarthritis of some years' standing. The results of this test, however, were entirely negative.

RAPID SEROLOGICAL METHOD OF IDENTIFYING CULTURES OF ERYSIPELOTHRIX RHUSIOPATHIAE

In determining the biological characteristics of cultures of *Erysipelothrix rhusiopathiae* obtained from suspected cases of swine erysipelas or received at the laboratory for identification, the cultures are always subjected to the agglutination test against known positive swine erysipelas serum.

The usual method of preparing an antigen from cultures of the erysipelas organism for the serological test requires a considerable amount of work and time. The following is a simple method of procedure in testing cultures, which is timesaving and which has given entirely satisfactory results in the writers' identification of the erysipelas organism.

A transfer of the culture to be studied is made from a fresh broth culture to a serum agar slant, which is incubated for 24 hours. The culture is then washed off the agar slant with $\frac{1}{2}$ to 1 cc of sterile normal salt solution. The quantity of salt solution used must be limited in order that the suspension may be sufficiently heavy for a satisfactory plate test. The entire growth of the organism is removed from the surface of the agar by means of a platinum loop and thoroughly suspended in the small quantity of salt solution at the bottom of the agar slant.

The plate and other equipment should now be made ready for the rapid test.

A 1 cc pipette is used for removing the suspension of organisms from the culture tube. Before the pipette is inserted in the culture tube a small, thin pledget of cotton should be folded over the tip of the pipette and securely wrapped around it to prevent the entrance of clumps of organisms, which would interfere with the proper interpretation of the test. After the pipette, containing a small quantity of the suspension, is lifted from the culture tube, the cotton is carefully removed from the tip with forceps, while the finger is retained on the upper end of the pipette to prevent the outflow of the suspension.

Two drops of the suspension of organisms from the pipette are placed on the plate for testing, and one standard loopful of hyperimmune anti-swine-erysipelas serum is added. If the organism is *Erysipelothrix rhusiopathiae*, a marked rapid clumping will take place. Organisms other than *E. rhusiopathiae* fail to cause any agglutination.

SUMMARY

Eight pigs were exposed to swine-erysipelas infection by injections of cultures of *Erysipelothrix rhusiopathiae*. Six of the pigs which showed clinical symptoms of swine erysipelas developed agglutinins in the blood detectable from the third to the sixth day after inoculation, thus indicating that the agglutinins make their appearance early in the disease, or within a brief period after the first clinical evidence of the disease is noted. The other two pigs, 3 weeks of age, failed to show any clinical evidence of infection, and the blood of these pigs remained consistently negative to all plate and tube tests for swine erysipelas, made on various dates subsequent to the time of exposures.

Blood serum from a pig which showed typical diamond skin lesions, or the urticarial form of swine erysipelas, and from which *Erysipelothrix rhusiopathiae* was recovered, gave negative results to both the plate and tube agglutination tests for swine erysipelas. Negative serological results have been reported in five other cases of typical diamond skin disease. The reason for the negative serological results in these cases has not been determined.

It has been experimentally demonstrated that the subcutaneous injection of 30 cc of anti-swine-erysipelas serum (equine) resulted in the passive transfer of agglutinins, which could be readily demonstrated within 24 hours by the plate and tube tests. The titer of the serum of the experimental pig, which was entirely negative prior to inoculation, indicates that a very large portion of the agglutinins in the specific serum had been transferred to the normal pig injected. Another pig, of approximately the same age and weight, injected

subcutaneously with 30 cc of normal horse serum remained consistently negative to both the plate and tube tests.

Blood serum from a human case, in which the history and clinical symptoms strongly indicated *Erysipelothrix rhusiopathiae* infection, gave positive reactions to both the plate and tube tests, the latter showing a titer of 1 to 1,000. After 4½ months agglutinins had apparently disappeared from this serum. A number of human sera from apparently normal individuals gave negative results to the test.

A rapid whole-blood plate test made in the case of a man affected with chronic polyarthritis gave negative results.

A description is given of the technic of a rapid serological method of identifying cultures of *Erysipelothrix rhusiopathiae*.

HISTOLOGICAL STUDIES OF RICE LEAVES INFECTED WITH HELMINTHOSPORIUM ORYZAE¹

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INTRODUCTION

The fungus causing helminthosporium leaf spot of rice (*Oryza sativa* L.) was named *Helminthosporium oryzae* by Brede de Haan (1)² in 1900. According to Nisikado and Miyake (6, p. 134): "The first authentic report of the helminthosporiose of rice plant is that of S. Hori [(4)], who in 1892 found the disease on the glumes of rice in the suburbs of Tokyo." Of subsequent contributions to the knowledge of this disease and the causal fungus the Japanese have made by far the greatest number.

In 1924 Ocfemia (7, 8) reported on the occurrence of the helminthosporium disease of rice in the southern part of the United States and the Philippine Islands and investigated the relation of soil temperature to germination of certain rice varieties and to infection by *Helminthosporium oryzae*. In 1927 Ito and Kuribayashi (5) found the ascigerous stage of the fungus in culture and described it as *Ophiobolus miyabeanus*, but they did not find it under natural conditions. In 1928 the writer found on naturally infected rice plants in the vicinity of Crowley, La., an ascomycetous fungus that apparently is morphologically identical with the ascigerous stage of *O. miyabeanus* as described by Ito and Kuribayashi. However, these ascospores, grown on artificial culture media, have not as yet produced *H. oryzae*, nor has *H. oryzae* produced the ascigerous stage in culture for the writer.

Except for minor differences, the manifestations of the disease as it occurs in the United States seem to be the same as elsewhere in the world. The lesions produced by the fungus are usually narrowly elliptical spots with grayish centers and brown margins. Various gradations are found from narrowly elliptical lesions in some varieties to circular spots in others. Typical leaf spots are shown in figure 1.

The fungus has a number of hosts. Suematsu and Okapa (9) reported 39 members of the grass family and Nisikado and Miyake (6) 25, some of which are the same as those listed by Suematsu and Okapa.

Many ways to control the disease have been suggested, but the most satisfactory, it seems, is the development of resistant varieties. In Japan several varieties have been reported as very resistant to the disease, and the writer has noted a wide range in the resistance of commercial varieties now grown in the lower Mississippi Valley. In the United States commercial varieties have been crossed with resistant varieties from other countries. It is hoped that resistant strains of commercial value may be isolated from these crosses.

¹ Received for publication Oct. 1, 1934; issued April 1935. Investigations conducted in cooperation with the Arkansas, Louisiana, and Texas Agricultural Experiment Stations.

² Reference is made by number (italic) to Literature Cited, p. 90.

The purpose of the present study was to determine the nature of resistance and susceptibility to *helminthosporium* leaf spot in rice varieties.

MATERIAL AND METHODS

Leaves of 12 varieties of rice infected naturally in the field were used in this study. These were the short-grain varieties Kameji, Butte, Bozu, and Aikoku; the medium-grain varieties Blue Rose, Early Prolific, and Shoemed; and the long-grain varieties Storm Proof, Honduras, Fortuna, Lady Wright, and a selection from a Patna rice, J-131. Of these, Kameji, Aikoku, Butte, and Shoemed show considerable resistance and Fortuna some resistance to the *helminthosporium* leaf spot. The other varieties named are susceptible.

The material, with the exception of J-131, was killed and fixed in the field with strong chromo-acetic fixative.³ The specimens were

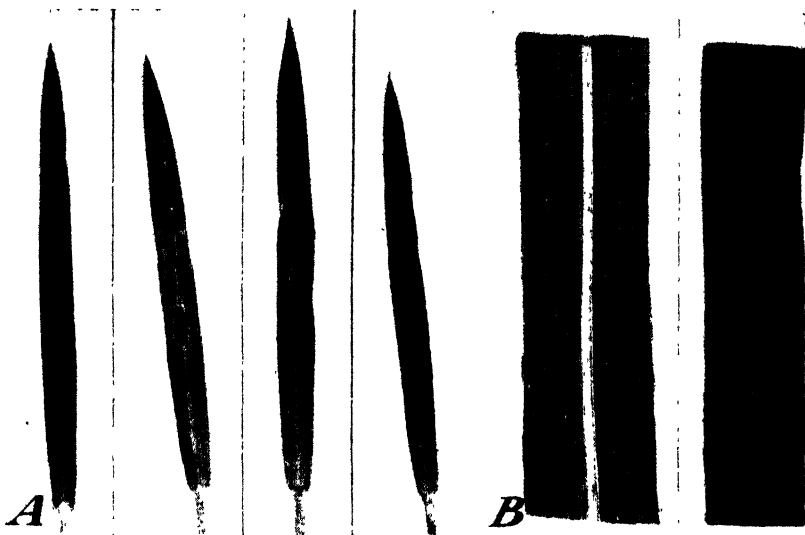


FIGURE 1.—Leaves of Blue Rose rice showing lesions caused by *Helminthosporium oryzae*: A, From artificial inoculations in greenhouse. $\times 0.85$ B, From natural infections, Beaumont, Tex. $\times 1\frac{1}{4}$

allowed to remain in the killing solution from 24 to 36 hours before being washed. As soon as the killing solution was washed out, the specimens were treated for 20 to 30 minutes with concentrated hydrofluoric acid to remove the silicon from the epidermis, and again washed.

The specimens were then dehydrated in ethyl alcohol up to 70 percent, after which a modification of Zirkle's (10) butyl alcohol-ethyl alcohol series was used as follows: From 70-percent ethyl alcohol the specimens were put into a 25-percent solution of *n*-butyl alcohol in 95-percent ethyl alcohol. After 2 hours this was drained off and a solution of 50-percent *n*-butyl alcohol in 95-percent ethyl alcohol was added, and 2 hours later 75-percent *n*-butyl in 95-percent ethyl alcohol. The specimens were allowed to remain in this solution overnight and were then changed to *n*-butyl alcohol. The remainder

³ Chromic acid 1 g, acetic acid 1 g, water 100 cc

of the schedule is the same as suggested by Zirkle (10). The specimens were then embedded and cut. The cross sections were cut 3μ or 6μ and the longitudinal and tangential⁴ sections were cut 10μ . Sectioned material was stained in iron-alum haematoxylin and methylene blue. The former stain also was used for portions of leaves stained in toto.

ANATOMY OF HEALTHY LEAVES

The salient features of the anatomy of a rice leaf are shown diagrammatically in figure 2. The parenchyma consists of the armed-type cells referred to by Haberlandt (3, pp. 277-278) in *Bambusa*, *Arundinaria*, *Elymus*, *Calamagrostis*, and *Alistromeria*. This same type of

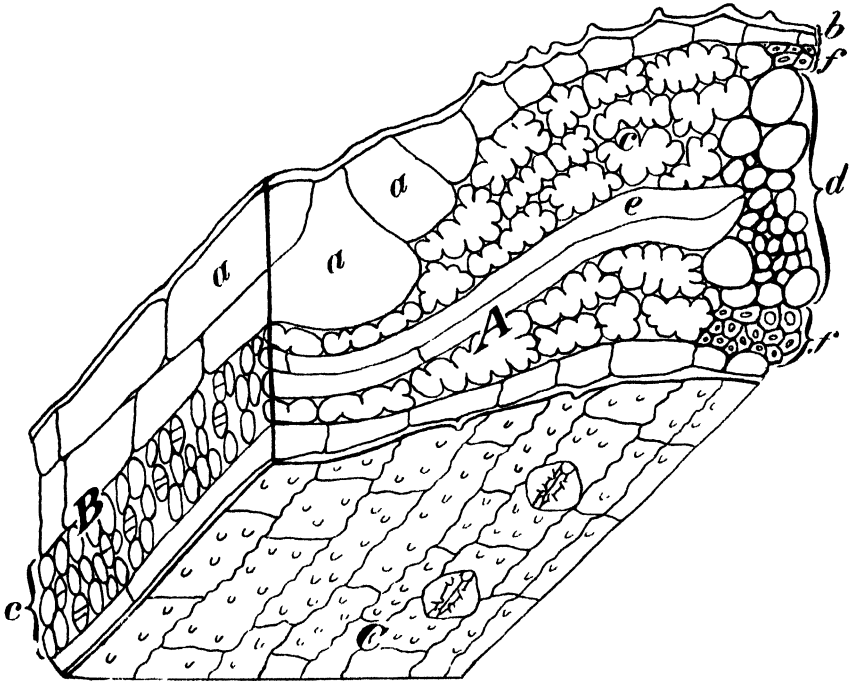


FIGURE 2 Diagram of a portion of a rice leaf showing (A) cross and (B) longitudinal section and (C) surface view of lower epidermis. a, Motor cells, b, upper epidermis with papillae, c, armed-type parenchyma cells, d, fibro-vascular bundle; e, transverse bundle, f, sclerenchyma cap. $\times 560$

parenchyma was also found by the writer in *Arundinaria gigantea* (Walt.) Chapm. As shown in figure 2, these parenchyma cells in most cases lie with their long axes crosswise of the leaf.

In cross sections of the leaf the armed-type parenchyma cells are, in general, irregularly rectangular in outline with invaginations at the periphery. In this view their short axes range from 7μ to 15μ and their long axes from 15μ to 40μ (figs. 2, 3, A, B).

In longitudinal sections of the leaf the armed-type parenchyma cells are rectangular to elliptical in outline, depending on the portion of the cell cut and the location of adjacent cells. In this view their

⁴ Longitudinal section, as here used, denotes a section cut through the leaf at right angles to its surface and parallel to its long axis; tangential section, one cut parallel to its surface.

short axes range from 4μ to 10μ and their long axes from 8μ to 20μ . In cases where the cells are cut near the periphery and through the

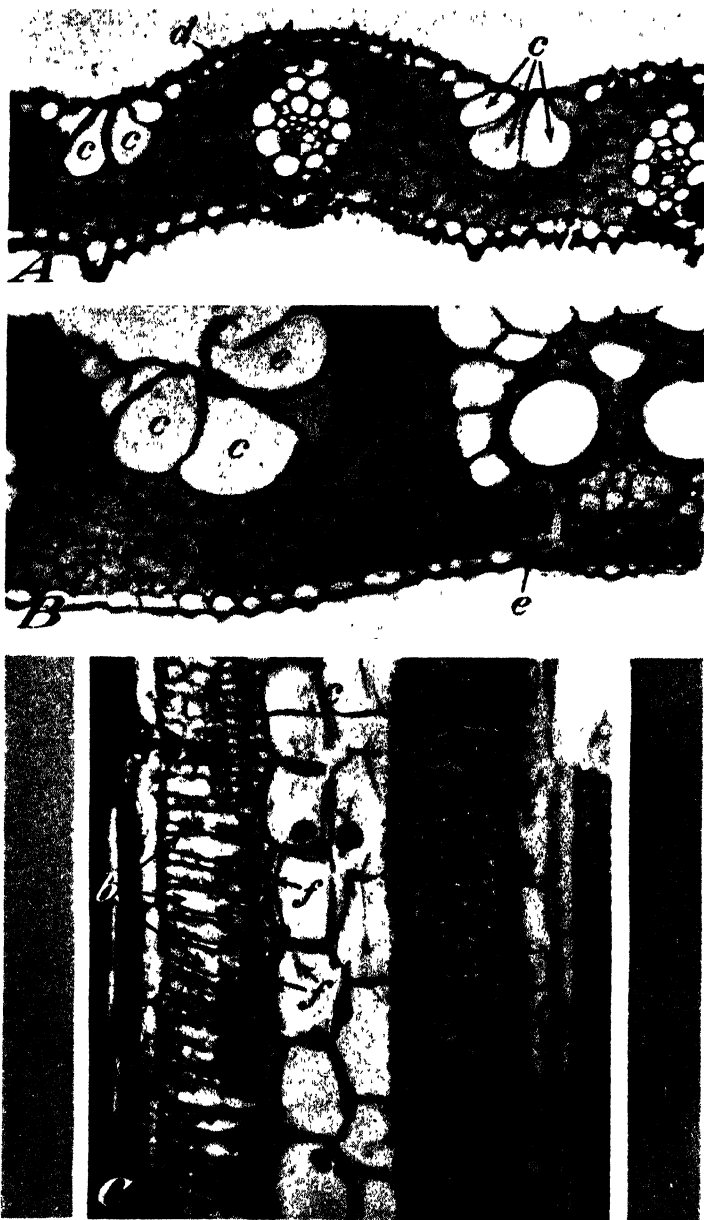


FIGURE 3.—Cross section of portion of leaf of (A) Shoemed and (B) Blue Rose rice, and (C) tangential section of portion of leaf of Blue Rose rice. *a*, Armed-type parenchyma cells; *b*, intercellular spaces; *c*, motor cells; *d*, small bundle; *e*, large bundle; *f*, hyphae of *Helminthosporium oryzae*. All $\times 288$.

invaginations there appear to be numerous small cells. Examination of cross sections, however, shows that what appear to be cross walls

of small cells (fig. 3, *C*, *a*) are the invaginations of relatively large cells shown in cross sections in figure 3, *A*, *B*.

In cross sections of the leaves, as shown in figure 3, *A*, *B*, there are no conspicuous intercellular spaces. Only small openings occur

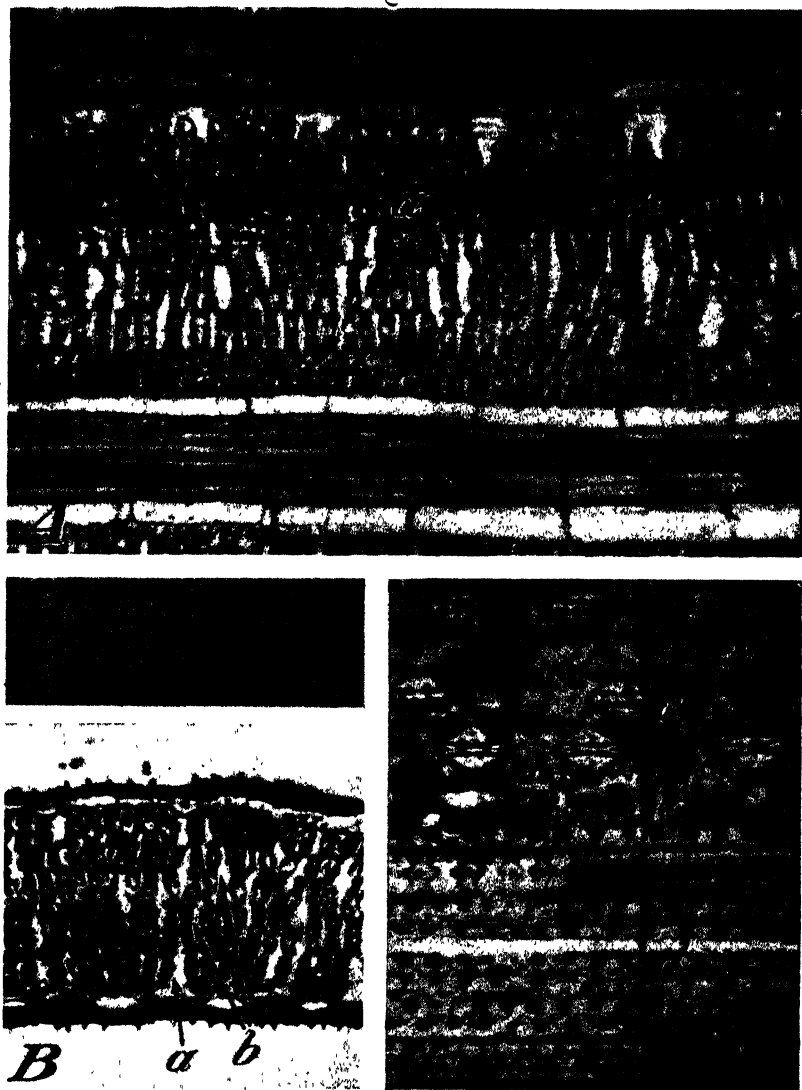


FIGURE 4. —Tangential (*A*) and longitudinal (*B*) sections of portions of leaves of Shoemed and Blue Rose rice, respectively, showing substomatal chambers (*a*) and intercellular spaces (*b*); and surface view (*C*) of portion of leaf of Shoemed rice showing lower epidermis covering parenchyma (*c*) and a large bundle (*d*). All $\times 320$.

opposite the invaginations. There are, however, rather large substomatal chambers which are more evident in longitudinal and tangential sections (fig. 4, *A*).

In longitudinal and tangential sections intercellular spaces are abundant (fig. 3, *C*, *f*). Frequently they extend from the upper to the lower epidermis (fig. 4, *B*) and occur in bands on each side of the bundles.

Considerable difference was found in the size of the intercellular spaces of various rice varieties when examined in longitudinal sections. In Aikoku the intercellular spaces are small and relatively infrequent. In Kameji, Bozu, and Butte they are somewhat larger and occur more often. In Shoemed, Blue Rose, Early Prolific, Storm Proof, and J-131 they are still larger, and the largest were found in Fortuna and Honduras.

The stomata occur in parallel rows on the upper and lower surfaces of the leaf. The rows of stomata alternate with the bundles and motor cells on the upper surface. The bands of stomata on the lower surface (fig. 4, *C*, *d*) are opposite those on the upper surface.

The substomatal chambers are relatively large and connect with the intercellular spaces just referred to and, together with the stomata, constitute the system for the exchange of gases in the photosynthetic area of the leaf.

Most of the epidermal cells and the guard cells of the stomata are studded with papillae as shown in figures 2; 3, *A* and *B*; 4, *A* to *C*; 5, *A* and *E*; and 6, *A* to *C*. These papillae differ considerably in size, shape, and distribution as shown in the illustrations. Those on the epidermal cells extend approximately at right angles to the epidermal surface, while those on the guard cells of the stomata are inclined toward the stomatal opening. On the upper and lower epidermis there occur several kinds of hairs.

ANATOMY OF DISEASED LEAVES

The symptoms of helminthosporium leaf spot of rice, as it has been observed by the writer, coincide with the descriptions of previous investigators. Lesions have been found on plants 18 hours after inoculation, as reported by Farneti (2) and others. In the lesions that are just appearing, the cells in the vicinity of the invading mycelium are brownish in color and the cell walls have partly collapsed. The invading mycelium from an appressorium may enter the leaf through a stoma or penetrate directly into the cells of the epidermis, as reported by Nisikado and Miyake (6) and Ocfemia (7, 8). It appears from the specimens examined that invasion is most frequent in the motor cells, and often the invaded motor cells are almost filled with the mycelium of the fungus. Such a condition is shown in figure 5, *A*, *B*, on a leaf of Kameji and Bozu, respectively. Hyphae in the motor cells are shown in figure 5, *A* to *C*. The section shown in figure 5, *C*, is from a portion of a leaf of Lady Wright inoculated in the greenhouse. This portion of the leaf was stained in toto to show how the hyphae have invaded the motor cells. Toxic substances produced by the fungus soon cause discoloration and death of the neighboring armed-type parenchyma cells.

Lateral branches of the mycelium that penetrate the inner walls of the motor cells are produced, and in this way the mycelium gains entrance into the intercellular spaces of the photosynthetic area of the leaf. Two systems of mycelium are then produced. One develops just beneath the epidermis and spreads through substomatal cavities

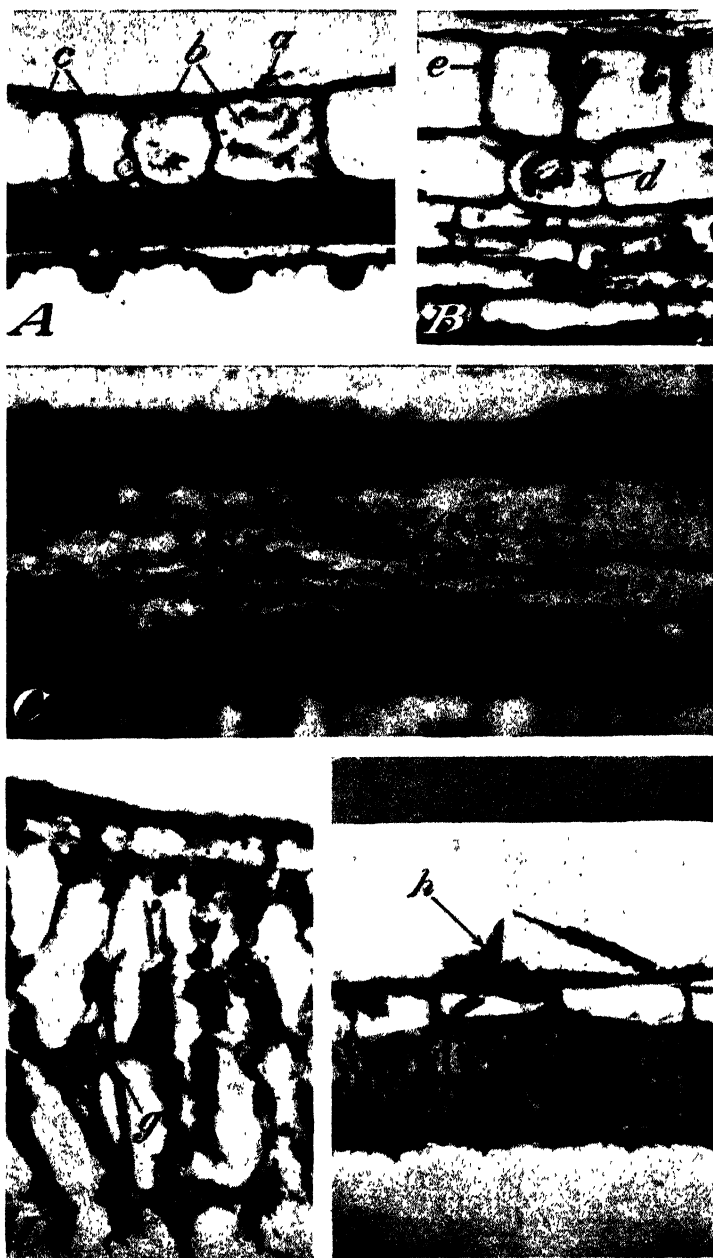


FIGURE 5. --A, Longitudinal section of a portion of a leaf of Kameji rice, showing remains of spore on surface of motor cell at *a*, two motor cells nearly filled with mycelium at *b*, and intracellular hyphae at *c*. $\times 288$. B, Tangential section of a portion of a leaf of Bozu rice, showing a cell almost filled with mycelium of the fungus at *d*, and intracellular hyphae at *e*. $\times 288$. C, Surface view of a portion of a leaf of Lady Wright rice, showing hyphae of the fungus in the motor cells at *f*. $\times 228$. D, Longitudinal section of a portion of a leaf of Storm Proof rice, showing an intercellular hypha at *g* in an intercellular space. $\times 900$. E, Longitudinal section of a portion of a leaf of Blue Rose rice, showing conidiophores, at *h*, beginning to form on an infected motor cell. $\times 288$.

from one system of intercellular spaces to the next. The other consists of branches from this system that invade the intercellular spaces between the faces of the armed-type parenchyma cells. A lateral strand of mycelium invading the intercellular space of a leaf of Storm Proof is shown in figure 5, *D*, *g*.

The most susceptible varieties have large intercellular spaces and large substomatal chambers, invasion progresses rapidly, and the leaf spots frequently extend from the midrib of the leaf to the margin. The spots are at times circular but more often narrowly elliptical. In many cases several lesions coalesce to produce large spots.

In areas of active invasion the host cells first become yellowish in color, the chloroplasts disappear, the protoplasm dies, and the cells partially collapse.

The bundles tend to form barriers against the lateral spread of the fungus. To invade adjacent areas beyond a bundle it is necessary that the fungus penetrate the bundle sheath. Apparently this is accomplished much more easily in some varieties than in others. In very resistant varieties it apparently does not occur.

In resistant varieties invasion of the motor cells occurs, but in most cases the mycelial strands are small and soon become brownish in color and thick-walled with rather sparse contents, whereas those found in susceptible varieties are not brownish in color and are large in diameter and rich in cytoplasm, indicating much greater vigor. The conidiophores are formed from a plexus of the mycelium in the intercellular spaces or in the motor cells. The latter case is shown in figure 5, *E*. Five immature conidiophores can be seen in this group.

In resistant varieties a deposit is formed in the intercellular spaces that aids in restricting the fungus to the area of primary invasion. This ability of the parenchyma cells of an invaded area to protect those of adjoining areas from invasion is apparently the greatest factor in resistance in the leaves. These deposits are found most highly developed in Shoemed, but they also have been found to some extent in susceptible varieties. In the more susceptible varieties they are formed by groups of cells but never in sufficient numbers to isolate a given area completely, as is the case in Shoemed. Changes in the walls of the armed-type parenchyma cells and in the cells of the bundle sheath, in the immediate vicinity of the mycelium are seen in sections stained with methylene blue even before the deposits are formed. The cell walls become thicker, stain more intensely with all stains used, and after a time become somewhat yellowish in color.

The deposits are formed between the armed-type parenchyma cells considerably in advance of any mycelium, discoloration, or disintegration of the host cells. The deposits are solid in the case of the very narrow intercellular spaces and are sometimes hollow in large intercellular spaces or in substomatal chambers (fig. 6.). In resistant varieties these deposits extend from the upper to the lower epidermis, from one bundle to the next, and along the bundles. In this way, openings that may exist from one set of intercellular spaces to the next are filled so completely that in case the fungus has gained entrance into the photosynthetic portion of the leaf no further internal spread is possible. These deposits also protect the bundles so that the fungus is unable to reach additional sources of food or to invade adjacent photosynthetic areas. The protoplasmic content of the

motor cells is very limited and as soon as the reserve food obtained from the area of primary invasion is exhausted the mycelium apparently dies for want of nourishment.

The chemical nature of the deposits is not known. In unstained sections they are yellowish brown. They stain intensely with iron-alum haematoxylin, as does the outer wall of the epidermis. When they were tested for cutin, suberin, or pectin, no positive reaction was secured. Because of the high silicon content of the epidermis it is necessary to treat all material with hydrofluoric acid before sectioning. This treatment may so alter the deposits that it is not possible to determine their composition in sectioned material.

Usually the formation of the deposits is first observed between the parenchyma cells near the bundles just under the upper and lower epidermis.

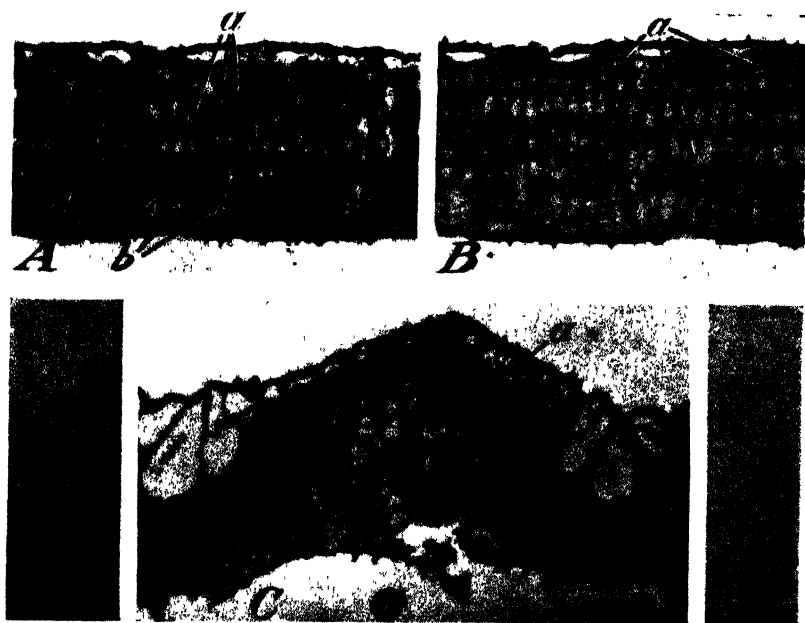


FIGURE 6.—Longitudinal (A and B) and cross (C) sections of portions of infected leaves of Shoemed rice, showing substomatal chambers and intercellular spaces filled with deposit at *a*. In the large intercellular spaces shown at *b* the deposits are hollow. All $\times 288$.

SUMMARY

The anatomy of healthy rice leaves is characterized by papillate epidermis, motor cells, armed-type parenchyma cells, and usually by large intercellular spaces in photosynthetic areas.

In rice leaves affected with leaf spot caused by *Helminthosporium oryzae* the hyphae of the fungus grow intercellularly in the photosynthetic areas and intracellularly in the motor cells and bundle sheath.

The bundle sheaths of resistant varieties are less readily penetrated by the fungus than are those of susceptible varieties. The bundles interfere with the lateral spread of the fungus in the leaf.

In resistant varieties of rice the invading fungus is hemmed in by the formation of deposits, which accumulate in the intercellular spaces about an infection. The chemical nature of these deposits has not been determined.

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GNOMONIA NERVISEDA, THE PERFECT STAGE OF THE FUNGUS THAT CAUSES THE VEIN SPOT DISEASE OF PECAN FOLIAGE¹

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INTRODUCTION

The vein spot disease of the pecan (*Hicoria pecan* (Marsh.) Britton)³ was collected by Demaree⁴ near Austin, Tex., in 1920 and again at Ferriday, La., in 1929. The writer has collected the disease on both wild and cultivated pecans in Arkansas, Louisiana, Mississippi, and Texas.

THE DISEASE

In the vicinity of Shreveport, La., vein spot first appears in May or June on the foliage of pecan trees, and in some orchards it causes the leaves of certain varieties to shed prematurely. The disease may attack the rachis, the petiole, or the veins, but the entrance of the fungus is always confined to the vascular system of the leaf. Infection may take place at or near the end of the midrib of a leaflet and extend down the midrib to its base, producing a narrow black necrotic streak seldom extending more than 2 or 3 mm on either side of the midrib. The infection may also extend from the main vascular bundle that runs lengthwise to the smaller lateral ones. When numerous small lateral bundles are killed, the leaf tissue between them dies, forming large dead areas that may later involve the entire leaflet, causing it to drop.

The fungus may attack either the terminal or the basal portion of the rachis and gradually extend to the opposite end, causing the leaflets to fall one at a time until finally the rachis, devoid of leaflets, is left hanging on the tree. In other instances, when either primary or secondary veins become infected, the fungus does not spread extensively but causes a small oval-to-circular black spot. The leaflet may have 8 or 10 of these black spots, which sometimes are not over 2 mm in diameter. The vein divides such spots into two almost equal parts. In some instances, the fungus attacks the midrib of one leaflet and causes it to abscise without interfering with the rest of the leaf, whereas in other instances it will attack the petiole and affect the entire compound leaf. In serious cases, the entire vascular system of many leaves is attacked and severe defoliation results.

Because of its striking similarity to pecan scab (*Cladosporium effusum* (Wint.) Demaree), vein spot has perhaps been confused with scab by growers and phytopathologists. Since scab spots no longer

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² The writer wishes to acknowledge the helpful services of the following members of the Bureau of Plant Industry: J. B. Demaree, for his criticism of the manuscript; C. L. Shear, for suggestions in the determination of the causal organism of the vein spot fungus; and Edith K. Cash, for assistance in translating the technical description into Latin.

³ *Carya pecan* Engl. and Graebn.

⁴ This information was furnished the writer through correspondence with J. B. Demaree, pathologist, Division of Fruit and Vegetable Crops and Diseases.

sporulating are black and are about the same shape and size as lesions of the vein spot disease and since scab spots also appear on the leaf veins, it is difficult by casual examination to differentiate between scab and vein spot lesions.

THE PATHOGENE

CONIDIAL STAGE

The conidial stage of the fungus causing the vein spot disease does not appear until the summer or early fall in the vicinity of Shreveport, La. The pycnidial bodies are usually most abundant about the middle of October. Immediately before the formation of the fruiting bodies, which are considered as dimidiolate pycnidia, the diseased areas on the ventral side of the leaf take on a grayish color (fig. 1, A, B, C). The pycnidia usually appear in these areas, either singly or in groups, and are difficult to see without some magnification.

In 1933 the writer¹ described the pycnidial stage of the fungus causing the vein spot disease as *Leptothyrium nervisedum*, n. sp. It was pointed out that leaves containing the pycnidial stage of the fungus were collected in October 1930 and placed outdoors in wire cages to overwinter. The leaves were examined at regular intervals of 3 to 4 weeks to ascertain whether any development was taking place. No change was observed, but spores were found to be viable as late as May 1 the following year. It was then thought that the fungus passed the winter in the pycnidial stage. More recent investigations have demonstrated the relationship between *L. nervisedum* and a perfect stage. Pecan leaves heavily infected with the vein spot organism in 1932 were observed the following April to be bearing numerous perithecia of the *Gnomonia* type. The perithecia were found only on the past year's lesions caused by the vein spot fungus.

The work was repeated the following year with leaves collected at Ferriday and Shreveport, La., with similar results except that perithecia were less abundant that year. For reasons that will be brought out later in this paper, the fungus is considered an undescribed species and the binomial *Gnomonia nerviseda* is suggested.

TECHNICAL DESCRIPTION

Gnomonia nerviseda, n. comb.

Peritheciis plerumque hypophyllis, ellipsoideis, singulis vel gregariis, pycnidiiis *Leptothyrii nervisedi* intermixtis, 250 μ -275 μ latis, 160 μ -185 μ altis, rostris 200 μ -300 μ longis, 75 μ -100 μ latis praeditis; muris peritheciiorum ex strato 2-3 cellarum rufo-brunnearum vel nigrarum, muris rostrorum ex strato 5-8 cellarum similarium sed longiorum angustiorumque compositis; ascis tenuiter tunicatis, hyalinis, cylindraeceis, 36 μ -42 μ \times 8 μ , apice poro munitis, ascosporis 14 μ 15 μ \times 4 μ -5 μ , curvatis, guttulatilis, uniseptatis, ad septum constrictis, utrinque appendicula gelatinosa auctis, paraphysibus nullis.

Pycnidiiis, hypophyllis, depressis-pulvinatis, 35 μ 95 μ latis; conidiophoris simplicibus, septatis, rectis vel leniter curvatis, 14 μ 18 μ longis, 2 μ 4 μ latis; conidiis irregularibus interdum curvatis, oblongisovatis, continuis, 8 μ 13 μ longis, 2 μ -3 μ latis.

In foliis *Hicoriae pecan* (Marsh.) Britton, Arkansas, Louisiana, Mississippi, et Texas.

Perithecia mostly hypophyllous, ellipsoidal, single or gregarious, intermingled with pycnidia of *Leptothyrium nervisedum*. The perithecia are 250 μ -275 μ wide by 160 μ -185 μ high. The beaks measure from 200 μ -300 μ long by 75 μ -100 μ wide. The walls of the perithecia are 2 to 3 cells thick while those of the beaks are 5 to 8 cells thick, but the cells of the beak are much longer and narrower than those of the perithecia. The cells of both the perithecia and beaks are reddish brown to

¹ COLE, J. R. VEIN SPOT OF THE PECAN CAUSED BY LEPTOTHYRIUM NERVISEDUM, N. SP. Jour. Agr. Research 46: 1079-1088 illus., 1933.

black in color. Asci, thin-walled, hyaline, cylindrical, 36μ - 42μ by 8μ with a pore at the apical end. Ascospores, 14μ - 15μ by 4μ - 5μ , curved, guttulate, 1 septate, constricted at septum and with gelatinous appendages at each end. Paraphyses not present.

Pycnidia hypophyllous, depressed pulvinate, 35μ - 95μ wide, conidiophores simple, septate, straight or slightly curved, 14μ - 18μ by 2μ - 4μ ; conidia irregular, sometimes curved, oblong to ovate, hyaline, nonseptate, 8μ - 13μ by 2μ - 3μ .

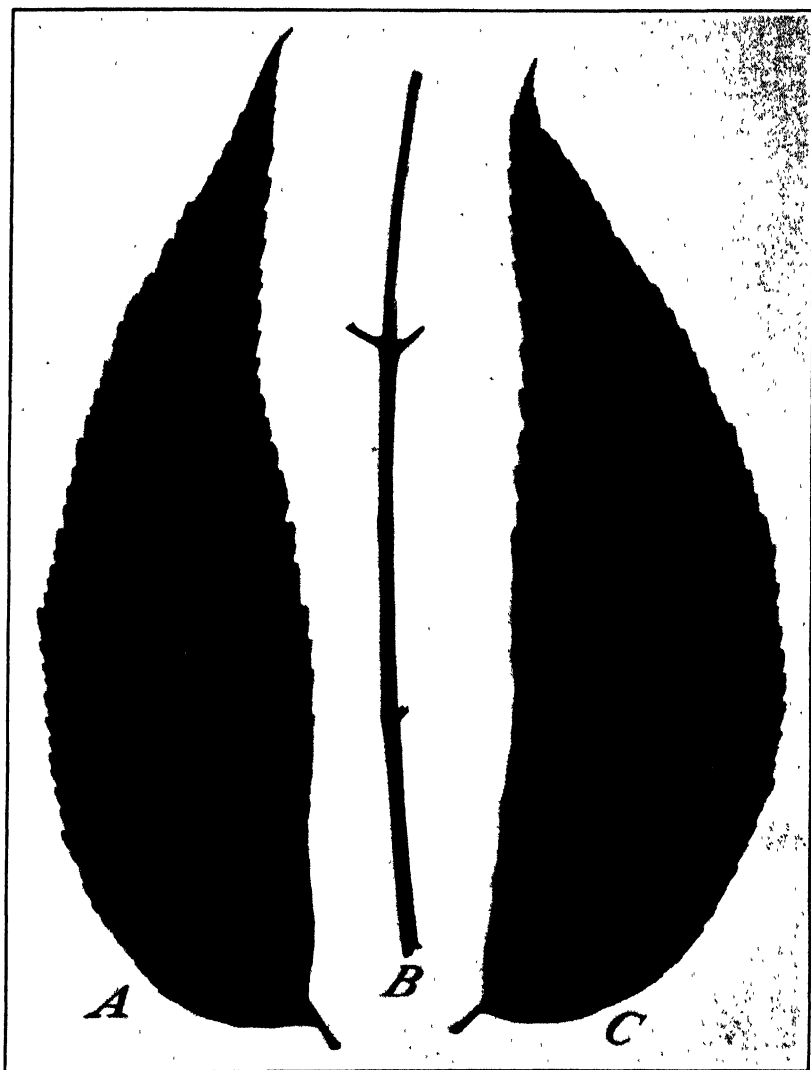


FIGURE 1. A and C, Pecan leaflets showing pycnidia on midrib; B, rachis discolored by vein spot fungus.

Lesions occur only on the vascular system of foliage of *Hicoria pecan* (Marsh.) Britton. The fungus has been found in Arkansas, Louisiana, Mississippi, and Texas.

Type specimens have been deposited in the pathological collections of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

PATHOGENICITY

Ascospore cultures were made from the *Gnomonia* perithecia and the resultant growth was identical with that made from the conidia of *Leptothyrium nerrisedum* Cole. Pycnidialike bodies, containing conidia, were produced in the ascospore cultures, but no perithecia were formed.

In May 1933, pecan leaves of the Van Deman variety were inoculated with ascospores from leaves collected at Ferriday, La.; with conidia from ascospore cultures; and with conidia from pycnidia from leaves collected in 1932. The results are shown in table 1.

TABLE 1.—Results of inoculating leaves of the Van Deman variety of pecan with conidia and ascospores of the vein spot fungus

[50 inoculations were made for each test]				
Series no. and date of inoculation	Inoculum	Infections	Date of reading	Remarks
		Num-ber		
1 (May 10) -----	Conidia from leaves.....	37	May 30	{ All inoculations were made on the leaf veins, both immature and mature
	Conidia from ascospore cultures.....	44		
	Ascospores from leaves.....	30		
	Check.....	2		
2 (June 8) -----	Conidia from leaves.....	29	June 28	{ Leaves were inoculated with no apparent difference in the susceptibility
	Conidia from ascospore cultures.....	36		
	Ascospores from leaves.....	19		
	Check.....	8		
3 (July 1)....	Conidia from leaves.....	34	July 21	
	Conidia from ascospore cultures.....	41		
	Ascospores from leaves.....	14		
	Check.....	10		

The vein spot disease was produced from all three series of inoculations. Infections appeared in 2 to 3 weeks after the inoculations were made, with no noticeable difference in the time required for infection to take place from ascospores or conidia.

Several species of fungi belonging to the genus *Gnomonia* have been reported on the pecan, but the spores of only two of them have appendages. Ellis and Everhart⁶ described one species, *Gnomonia setacea* (Pers.) Ces. and DeNot., var. *macrospora* Ell. and Ev., occurring on fallen leaves of *Quercus*, *Castanea*, and *Carya* (*Hicoria*) near Newfield, N. J., as having setae, but an examination of the type specimens by the writer disclosed that the ascospores either were immature or else had deteriorated so much that a fair comparison could not be made. However, the perithecia of the above fungus are not confined to any part of the leaves, being scattered over the entire leaf surface, and there was no evidence of old *Leptothyrium* pycnidia among the perithecia. The description of the above organism, according to Ellis and Everhart, indicates that it is different from the *Gnomonia* associated with the vein spot fungus. They describe *G. setacea* var. *macrospora* as being 4- to 8-spored and septate in the middle without constriction. The asci of *G. nerriseda* always contain 8 spores and they show a constriction at the septum (fig. 2, F).

Matz⁷ described a *Gnomonia* on living pecan leaves in Florida in 1917. Since the asci of this species contain usually 2 and rarely 1, 3,

⁶ ELLIS J. B., and EVERHART, B. M. THE NORTH AMERICAN PYrenomycetes. A CONTRIBUTION TO MYCOLOGIC BOTANY. p. 326. Newfield, N. J. 1892.

⁷ MATZ, J. AN UNDESCRIBED GNOMONIA ON PECAN LEAVES. Fla. Agr. Expt. Sta. Ann Rept 1917-89R-94, illus. 1918.

or 4 spores, variable in size, and since it is not known to be connected with a conoidal stage, it is considered by the writer as a separate species from *Gnomonia nerviseda*.

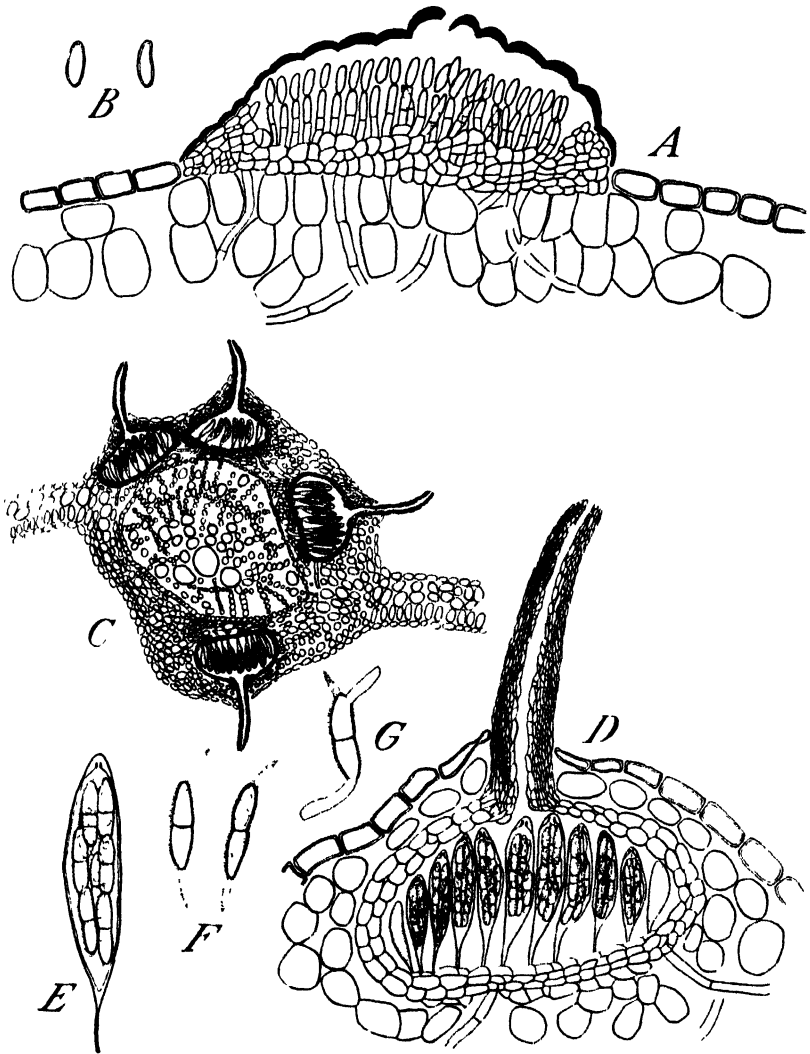


FIGURE 2 The vein spot fungus. A, Cross section of a leaf with a pycnidium on the midrib, $\times 400$. B, Representative conidia, $\times 800$. C, Cross section of a leaf vein, in early spring, showing the perithecia on both ventral and dorsal sides of the leaflet; however, the majority are on the lower or ventral side, $\times 100$. D, Typical perithecium from section of midrib, $\times 400$. E, Representative ascus showing typical shape, $\times 900$. F, Representative ascospores enclosed in gelatinous membrane, $\times 1,200$. G, Ascospore germinating after 24 hours, $\times 1,200$.

Wolf⁸ described *Gnomonia caryae*, the perfect stage of *Leptothyrium caryae* (Ell. and Ev.) Cole (*Gloeosporium caryae* Ell. and Dearn.), on hickory leaves (*Carya ovata* (Mill.) K. Koch), but the perithecia of this fungus are scattered over the entire leaf surface with spores 25μ

⁸ WOLF, F. A. A NEW GNOMONIA ON HICKORY LEAVES. Ann. Mycol. 10: 488-491, illus. 1912.

to 33μ long and 5μ wide and have no appendages, whereas the spore of *G. nerriseda* average 14μ to 15μ long and 4μ to 5μ wide and are enveloped in a gelatinous substance drawn out to a point resembling setae.

Cole⁹ described *Gnomonia caryae* var. *pecanae*, nov. var., on the pecan (*Illicia pecan*), but this fungus is morphologically different, both microscopically and macroscopically, from *G. nerriseda*. The spores of both species bear gelatinous appendages, but those of *G. caryae* var. *pecanae* are 22μ to 28μ long and 3μ to 5μ wide and perithecia measure 300μ to 350μ wide and 150μ to 250μ high, excluding the beaks. The spores of *G. nerriseda* are 14μ to 15μ long and 4μ to 5μ wide, with perithecia 250μ to 275μ wide and 160μ to 185μ high, excluding the beaks. Conidia from ascospore cultures of *G. caryae* var. *pecanae* produced lesions typical of that formed by the conidial stage of the liver spot fungus, *Leptothyrium caryae* var. *pecanae*, while conidia from ascospore cultures of *G. nerriseda* produced lesions typical of the conidial stage of the vein spot fungus, *L. nerrisedum*.

SUMMARY

Vein spot, a foliage disease of pecans, *Illicia pecan* (Marsh.) Britton (*Caryae pecan* Engl. and Gracbn.), caused by *Gnomonia nerriseda*, n. comb., has been collected by the writer in Arkansas, Louisiana, Mississippi, and Texas.

The disease attacks the rachis, the petiole, or the veins, and is always confined to the vascular system of the leaf, thereby suggesting the name "vein spot." Heavy infection results in severe injury to the foliage or in premature defoliation.

The pycnidial stage of the vein spot fungus, *Leptothyrium nerrisedum* Cole, does not appear until summer or early fall, while the perfect stage appears the following spring on the fallen leaves along the old vein spot lesions.

The writer was able to demonstrate the relationship between the pycnidial and the perfect stage of the vein spot fungus.

The perfect stage of the vein spot fungus is described as new, *Gnomonia nerriseda*, distinct from *G. setacea* var. *macrospora* Ell. and Ev., from a two-spored *Gnomonia* described by Matz in Florida, from *G. caryae* Wolf, and from *G. caryae* var. *pecanae* Cole.

⁹ COLE, J. R. LIVER SPOT DISEASE OF PECAN FOLIAGE CAUSED BY *GNOMONIA CARYAE* *PECANAE*, NOV. VAR. Jour. Agr. Research 47, 869-881, illus. 1933.

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A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT¹

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LITERATURE REVIEW

Extensive studies of the morphological and anatomical development of the pecan nut (*Illicoria pecan* (Marsh.) Britton)³ have been reported by J. G. Woodroof (31),⁴ N. C. Woodroof (35), Woodroof and Woodroof (32, 33, 34), Shuhart (26, 27, 28, 29), Isbell (18), and Adriance (1). No systematic physiological study of the normal process of nut development has been reported. In fact, the only data known to the writers relative to the physiological changes that occur during the development of pecan fruits are some analyses of pecan kernels made at intervals during the filling process by Woodroof and Woodroof (33).

Numerous investigations relating to the effects of cultural and climatic conditions on the final quantity and quality of oil in many oily seeds have been reported. Fewer studies of the course of development of the oil and its constituents in seeds during the entire period are available. These are represented by the studies of Garner, Allard, and Foubert (15), Eyre and Fisher (10), Eyre (9), Bushey, Puhr, and Hume (5), Bloomendaal (3), Dillman (8), Johnson (21), Fomin (11), and Fuchs (12), dealing chiefly with annual crop plants such as flax, cotton, and soybean. These workers found that oil formation usually occurs rapidly during a relatively short period and often is practically complete some time before the seeds are fully mature.

The most complete study of oil-seed metabolism seems to be that reported in 1912 by Ivanow (19), who determined changes in carbohydrates, oil, oil constants, and nitrogen fractions during the development of the seeds of flax, rape, hemp, poppy, and sunflower. In most cases the period of seed development was represented by only 3 or 4 sampling dates. In all cases there was a period of intensive oil formation occurring near the middle of seed development. This was accompanied by a simultaneous but apparently independent accumulation of protein in the seed. Reducing-sugar content invariably decreased during the time when samples were taken, while sucrose showed increases in total amount in some cases but as a rule decreased in percentage concentration on a dry basis. Insoluble carbohydrate fractions usually decreased in percentage of dry weight, but the

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² The writers are indebted to L. D. Romberg, of the Division of Fruit and Vegetable Crops and Diseases, for assistance in the nitrogen determinations.

³ Synonym, *Carya pecan* Engl. and Graebn.

⁴ Reference is made by number (italic) to Literature Cited, p. 119.

actual amounts increased during the period of active growth, then remained relatively constant. There was no indication that pentosans in these oily seeds functioned as reserve materials or potential sources of oil. The various seed receptacles, insofar as they were studied, were found to lose sugars and nitrogen, especially soluble nitrogen, and to gain in percentage of insoluble carbohydrates, during the ripening process. In general, these results were considered to establish glucose as the precursor of oil formation in oily seeds. However, the major interest in Ivanow's studies concerns the chemical properties of the oil during its formation.

Studies on the development of oily seeds, in which attention was paid to constituents other than oil, seem to be relatively scarce. Perhaps the earliest work is that of Müntz (25), who determined sugars, starch, oil, and protein in rape seed and rape hulls at seven different stages in the development of the seed. After most of the oil was formed starch decreased and sucrose increased in the seed. Protein accumulated gradually during the ripening period. Since there was a notable decrease in the sugar content of the hulls and no important change in their low lipide content, Müntz concluded that these sugars were the principal source of carbon for oil formation and that the transformation took place in the seed as fast as the sugars were translocated. He also pointed out that, in general, immature oily seeds contain both reducing and nonreducing sugars, while the mature seeds contain only nonreducing forms. Leclerc du Sablon (22, 23) later presented data showing a decrease in the percentage of carbohydrate fractions in walnuts and almonds coincident with the increase in percentage of oil. However, he considered only the reducing-sugar fraction to undergo conversion to oil, since the absolute amounts of sucrose and of "amylose" were said to increase rather than decrease during the ripening process. Gerber (16, 17) found a rise in respiratory quotient above 1 during oil formation in olives and castor beans, indicating a conversion of carbohydrate to oil. Vallée (30) studied changes in sugar and oil content of almond kernels and pericarp. Since most of his data were expressed only on a fresh-weight basis, his interpretations are questionable. A decrease in percentage of sugars in the kernel on a dry basis preceding and during oil formation was considered as evidence that both reducing sugars and sucrose served as sources of the oil.

More recently Gallup (13, 14) and Caskey and Gallup (6) have studied quite extensively the chemical changes occurring in the cotton boll during its growth. Both the entire boll and the seeds showed gradual increases in the amounts of ash, crude protein, crude fiber, and oil from 16 days after blossoming until maturity. The percentage of nitrogen-free extract dropped rapidly, but, in terms of absolute amounts per boll or per seed, a similar decrease was not evident. In the lint there were decreases in percentage of all fractions except crude fiber, which increased rapidly. In terms of actual weight, nitrogen-free extract as well as crude fiber increased, while other constituents were irregular. Sugars determined from the twenty-first day to maturity, when oil formation was most active, decreased both in amount and in proportion to other substances in all parts of the boll. Lonzinger and Raskina (24), working with cottonseed, found a rapid increase in oil up to an age of 50 days.

Water-soluble material decreased for 35 days and then became constant; crude fiber increased for 40 days and then became constant; ash increased for 35 days and decreased after 50 days.

No study of the development of all major constituents in perennial oil-seed fruits, such as the pecan, has previously been reported. Such information on the physiological development of the pecan fruit is particularly desirable in order to understand fully the effects of various orchard conditions on the quality of the mature fruit or nut. A knowledge of the normal physiological development of various parts of the pecan fruit is necessary to an understanding of the effects of such factors as water shortage, defoliation by insect or fungus attack, shortage or abundance of mineral materials essential to the tree, and many other conditions that may develop in the orchard.

MATERIALS AND METHODS

Sample material was collected at intervals throughout the period from May 18 to November 14, 1932, from trees of the Burkett and Stuart varieties of pecan growing on the Pearce Farm near Austin, Tex. Table 1 gives a summarized description of the condition of the fruits on the various sampling dates, including the approximate number of weeks from full bloom and the average green weight per fruit. Samples collected on each date represented the average condition of the fruits for that date. All samples were taken from the same trees.

TABLE 1.—*Condition of pecan fruits on the several sampling dates*

BURKETT VARIETY			
Sampling date	Time after full bloom	Average green weight per fruit	Condition of material
	Weeks	Grams	
May 18.	5	0.075	Young nutlets with stigmatic surfaces quite black, hard, and dry.
June 30.	11	1.99	Apparently same as on May 18 except for size.
July 21.	14	7.69	Apparently same as on June 30 except for size.
Sept. 1.	20	32.35	Shucks firmly attached to shells; shells beginning to harden; kernels very watery and low in dry weight.
Sept. 12.	22	35.92	Shells quite hard; kernels not watery but very sticky.
Sept. 19.	23	33.34	Kernels doughy; red packing material very sticky.
Sept. 26.	24	38.50	Same as on Sept. 19.
Oct. 3.	25	38.29	Shucks loose from shells but not split along the four rays; shell markings developed.
Oct. 10.	26	38.09	Shucks loose from shells and many split along the four rays.
Oct. 17.	27	34.40	Shucks practically all split along the four rays; red packing material dried out considerably and more easily separated from kernel than previously.
Oct. 19 to Nov. 14.	27-31		Nuts apparently mature.
STUART VARIETY			
May 18.	5	0.078	Young nutlets with stigmatic surfaces quite black, hard, and dry.
June 30.	11	2.58	Apparently same as on May 18 except for size.
July 21.	14	8.68	Apparently same as on June 30 except for size.
Sept. 2.	20	32.14	Shucks firmly attached to shells; shells quite hard, kernels not watery but very sticky.
Sept. 19.	23	38.52	Kernels doughy; red packing material very sticky.
Sept. 26.	24	46.06	Shucks beginning to loosen from shells.
Oct. 3.	25	46.25	Shucks loose from shells and many split along the four rays; shell markings developed.
Oct. 10.	26	42.70	Shucks practically all split along the four rays.
Oct. 17.	27	38.87	Red packing material dried out considerably and more easily separated from kernels than previously.
Oct. 19 to Nov. 14.	27-31		Nuts apparently mature.

SAMPLING METHODS

The nut clusters were picked as rapidly as possible during the forenoon and brought to the laboratory in paper bags. The individual fruits were picked from the clusters, counted, and the whole lot weighed in order to determine the average weight per fruit at each sampling date. The whole fruits were ground in a food chopper, and, after thorough mixing, portions were weighed out for samples to be preserved by drying or by boiling in 80-percent alcohol. Beginning September 1, samples of separate tissues or fruit parts were taken in addition to the whole-fruit samples. The shuck was peeled and scraped away from the shell. The nut was then cracked and the kernel removed as completely as possible. The remaining material constituted the shell sample. All possible precautions were taken to prevent loss of material while the separations were being made. Record was kept of the number of fruits used for each separation (usually 50) and of the total weight of each type of tissue. Finally, each tissue was ground in a food chopper or a Nixtamal mill and portions were weighed out as for the whole-fruit samples.

The samples to be dried were killed by placing them in an oven at 100° C. for 1 to 2 hours. The others were transferred immediately to 500 cc Erlenmeyer flasks containing about 1 g of calcium carbonate with sufficient hot 95-percent ethyl alcohol to give a final alcohol concentration of at least 80 percent. The flasks were then closed with short-stemmed funnels and heated for approximately 30 minutes on a water bath kept at 90° to 95° C. The flasks were stoppered while still hot and stored in the dark until the samples were used for sugar analyses.

The kernels of the September 1 samples of the Burkett variety were still too watery to permit cracking off the shell after removal of the shuck. In order to remove the kernels without loss of the fluid they contained, the shuck and shell together were cut away from the kernel with a sharp knife, since it was found that leaving the shuck attached to the shell facilitated the separation. A similar lot of nuts was used from which the shucks were removed and sampled. The remaining shell and kernel portion was ground as a whole and sampled. From this combination analytical values for the shell alone could be calculated.

Beginning with the samples of September 19, it became practically impossible to make a perfectly clean separation between kernel and shell because of the very sticky nature of the red packing material between the two tissues.

ANALYTICAL METHODS

Whenever the nature of the material permitted, the official methods of the Association of Official Agricultural Chemists (2) were employed.

DRY MATTER.—Samples that had been weighed into aluminum cans and placed in ovens at 100° C. for 1 to 2 hours were transferred to a vacuum oven and dried to constant weight at a temperature of 80° and a pressure of less than 1 mm of mercury. The resulting dry material was ground in a drug mill or food chopper and stored in 8-ounce glass bottles. Kernel samples were kept in a refrigerator at 0° to 5°, the others at room temperature. The material was redried under the same conditions before it was used in further analyses.

TOTAL NITROGEN.—The procedure followed closely the Kjeldahl-Gunning-Arnold method except for the use of copper wire as the digestion catalyst, and the use of 30 cc of sulphuric acid rather than 25 cc because of the high oil content of some of the samples. In general, 2-g portions of dried material were used except for the oily kernels, in which case 1 g was more suitable.

ASH.—Samples of 2 to 5 g of the dried material were weighed into ignited and tared crucibles. Ashing was carried out in an electric muffle in which the temperature was raised slowly to a dull red heat, where it was held until ashing was complete.

OIL.—Twenty-five-gram samples of dried material were weighed into 100-cc beakers and transferred to alundum thimbles, the adhering oil being removed with the petroleum ether (b. p. 30–60) used to fill the large-size Soxhlet extractors. Extraction was continued for 8 hours, which time had been shown to be sufficient for removal of oil from pecan kernels. After the solvent had been distilled off the flasks and contents were dried for 4 hours in an air oven at 100° C., cooled in desiccators, and weighed. Deiler and Fraps (7) have reported that pecan oil when heated in a steam oven gained only 0.65 percent of its weight in 4 days, and it has been the experience in this laboratory that any increases in weight of oil due to oxidation during the 4-hour drying periods are insignificant. Since preliminary extractions of shuck and shell tissues showed that they contained relatively small amounts of lipide, it was considered sufficient to determine the oil content only of the kernel and whole-fruit samples.

FREE FATTY ACIDS.—The ether extracts of the kernel samples, amounting as a rule to 17 to 19 g, were titrated with alkali according to the official methods (2, p. 293 [32]). The results were calculated as percentage of oleic acid in the oil.

SUGARS.—The alcohol-preserved samples, representing as a rule 50 g of fresh material, were transferred to large Soxhlet extractors with 80-percent alcohol and extracted for 22 to 24 hours on a water bath kept at 95° to 99° C. The extracts of kernel and whole-fruit samples were transferred to separatory funnels, treated with 75 cc of chloroform, and diluted with water until a distinct chloroform layer separated. After standing overnight, the chloroform-lipide layer was run off and discarded. Such a chloroform extraction proved to be unnecessary for shell and shuck samples.

The alcohol extract was distilled almost to dryness in vacuo at temperatures not above 60° C. The residue was taken up with water, treated with sufficient saturated neutral lead acetate to clear the solution, and made up to a volume of 250 cc or 500 cc. Most pecan tissues require unusually large quantities of lead acetate for satisfactory clearing, 25 cc of saturated solution being necessary for all the samples except those of kernels, for which 10 cc was used. The cleared filtrate was deleded with solid anhydrous disodium phosphate.

The Munson-Walker method was used for the determination of sugars in aliquots of the lead-free filtrate. Reduced copper was determined by the volumetric permanganate method. For the determination of total sugars, inversion was accomplished by means of acid at room temperature as directed in official methods (2, p. 187, [23c]).

Since previous work had shown that the sugar in mature pecan kernels is almost exclusively sucrose, all reducing sugar values were calculated as invert sugar, and nonreducing sugars as sucrose.

POLYSACCHARIDES. The alcohol-insoluble residues were transferred to tared aluminum cans, dried in an air oven at 100° C., and weighed. After being ground in a drug mill they were stored in glass bottles and redried under the same conditions just before samples were weighed out for determination of polysaccharides. The dry residues from whole-fruit samples were extracted with petroleum ether to remove oil, and reweighed.

The official method for determination of starch in feeding stuffs by direct acid hydrolysis (2, p. 119 [21]) was applied to all residues except those from the kernel samples. The preliminary cold-water extractions were eliminated, since sugars had already been extracted with alcohol. The method could not be used satisfactorily on residues from the kernel samples because the acid seemed to dissolve enough of the high-protein content to interfere with the precipitation of cuprous oxide. No such difficulty appeared in the case of the whole-fruit samples, so the values for the kernel portion were calculated by difference from the data for whole-fruit, shuck, and shell samples.

Since the iodine-potassium-iodide test gave negative results on all types of preserved tissues, and since the diastase method for starch gave insignificant values on the first two whole-fruit samples as well as on representative shuck samples, the latter method was not used as was originally intended.

ANALYTICAL DATA

The accumulation of dry matter in the pecan fruit and several of its parts is shown in table 2. It is evident that the shuck and shell portions were structurally almost complete at the time the first separations were made. The rest of the analytical data are presented graphically in figures 1 to 14, which are self-explanatory. In all graphs the data for each type of tissue are consistently represented by the same kind of point mark, and the same base line is used throughout the series. The harvesting date, October 18, comes at 153 in the scale of days from the first sampling. Samples were not collected on the date of harvest, but were taken on the preceding and following days, so that the flow of materials from the tree into the fruit was free to continue for 1 day after the last preharvest sample.

The marked differences between curves obtained from data calculated as percentage of dry weight and those calculated as weight per fruit illustrate the importance in work of this type of knowing actual amounts of material in each tissue as well as the mere ratios of materials that the usual percentage values represent.

The close similarity in the graphs for the two varieties makes any distinction between them practically unnecessary in most cases.

TABLE 2 --Dry-weight changes in the pecan fruit

BURKETT VARIETY

Date	Days	Average dry weight per fruit of				Date	Days	Average dry weight per fruit of			
		Whole fruit	Shuck	Shell	Kernel			Whole fruit	Shuck	Shell	Kernel
	Number	Grams	Grams	Grams	Grams		Number	Grams	Grams	Grams	Grams
1932						1932					
May 18	0	0 024	-	-	-	Oct 17	152	14 58	4 55	1 41	6 22
June 30	43	58	-	-	-	Oct 19	154	-	-	1 04	5 39
July 21	64	1 99	-	-	-	Oct 21	156	-	-	4 00	5 45
Sept 1	106	8 27	3 61	3 91	0 54	Oct 24	159	-	-	3 96	5 50
Sept 12	117	10 52	4 29	4 14	1 46	Oct 26	161	-	-	3 87	5 40
Sept 19	124	11 23	4 00	4 06	3 39	Oct 28	163	-	-	1 01	5 48
Sept 26	131	13 24	4 31	4 31	4 38	Nov 4	170	-	-	1 00	5 45
Oct 3	138	13 92	4 33	4 14	5 58	Nov 14	180	-	-	3 93	5 46
Oct 10	145	15 01	4 42	4 28	6 12						

STUART VARIETY

May 18	0	0 025	-	-	-	Oct 17	152	15 19	4 20	5 45	5 28
June 30	43	68	-	-	-	Oct 19	154	-	-	5 09	5 43
July 21	64	2 08	-	-	-	Oct 21	156	-	-	5 67	5 16
Sept 2	107	9 45	3 48	5 05	0 94	Oct 24	159	-	-	5 65	5 27
Sept 19	124	12 52	4 04	4 97	3 65	Oct 26	161	-	-	5 77	5 33
Sept 26	131	14 46	4 54	5 48	4 36	Oct 28	163	-	-	5 70	5 17
Oct 3	138	14 53	4 40	5 21	4 84	Nov 4	170	-	-	5 82	5 37
Oct 10	145	14 75	4 19	5 19	5 18	Nov 14	180	-	-	5 82	5 38

MOISTURE AND DRY MATTER

Figures 1 and 2 show that the kernel and shell portions of the fruit were subject to a dehydration process from the time the watery kernel could first be removed whole until it was mature, although the surrounding shuck contained more than 75 percent moisture throughout the entire period. The increase in percentage of dry matter in kernel and shell followed roughly a double drying curve; the first, from September 1 until harvest; the second, from harvest on.

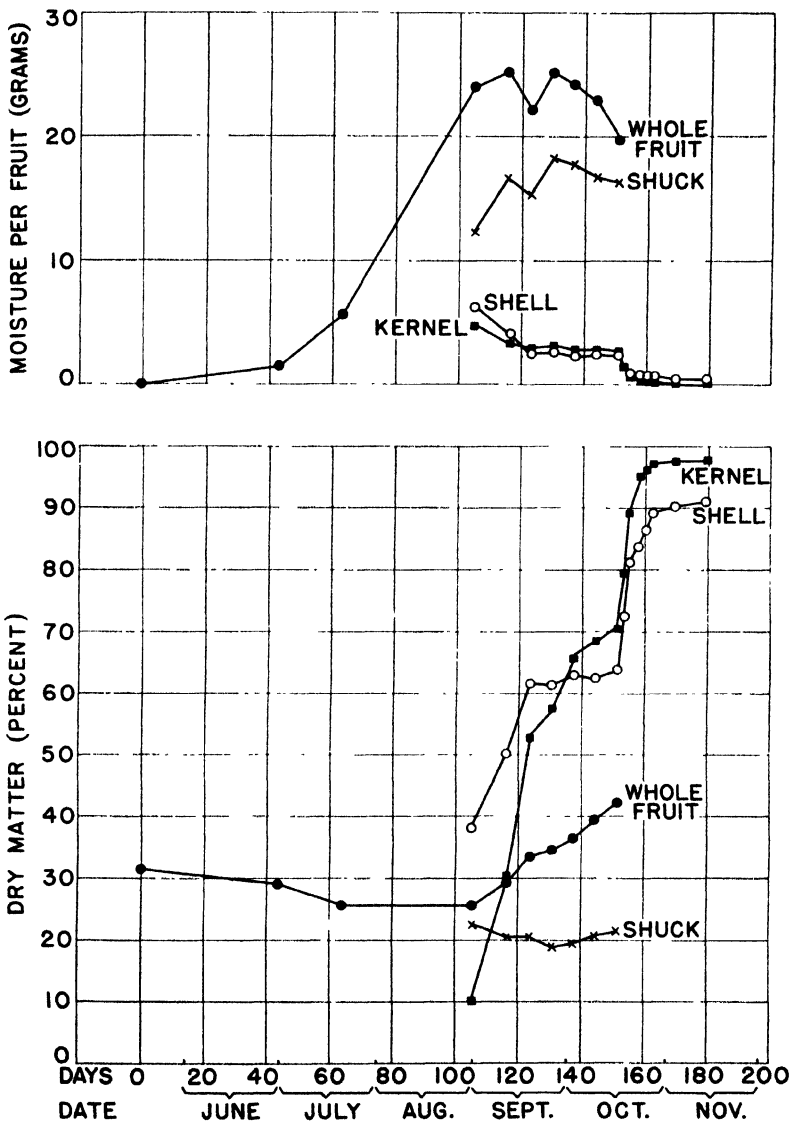


FIGURE 1.—Changes in dry matter and moisture content during development of the pecan nut (Burkett variety).

ASH

The amount of mineral matter in the whole fruit increased quite regularly from the beginning of its development until harvest. It is

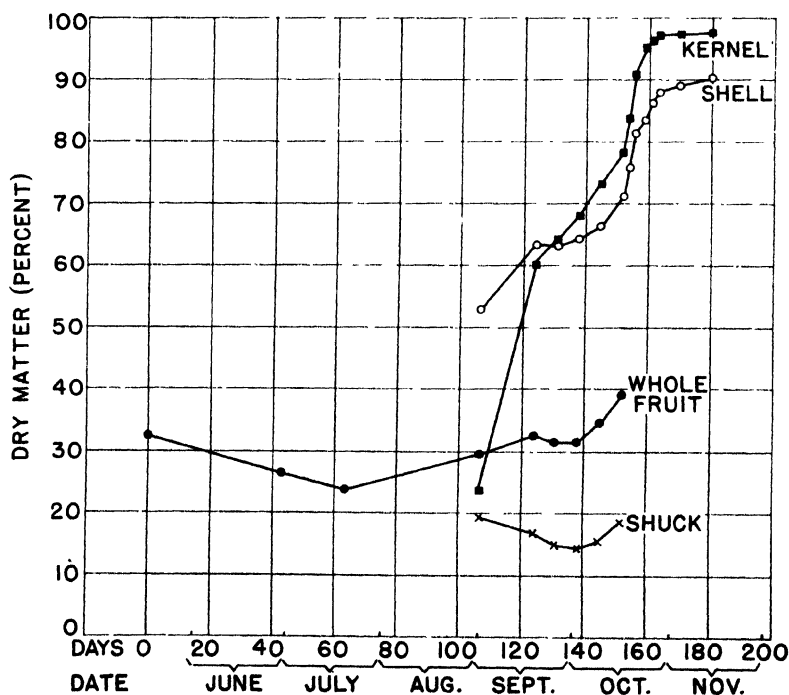
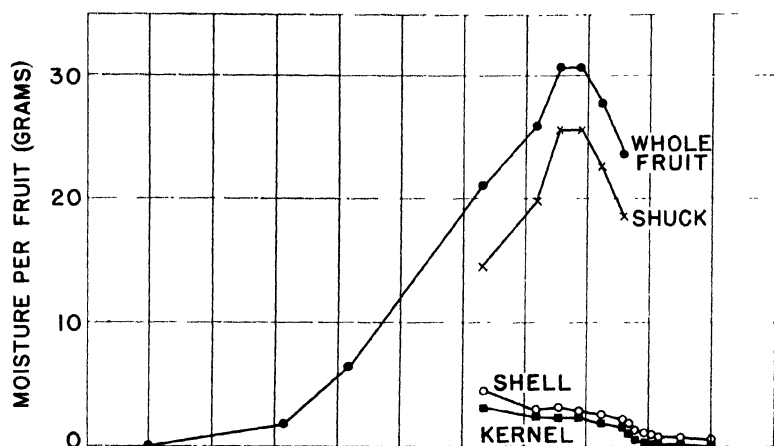


FIGURE 2.—Changes in dry matter and moisture content during development of the pecan nut (Stuart variety).

significant that the larger part of this accumulation occurred in the shuck, where the mineral constituents doubled in amount during the

relatively short filling period. The decrease in percentage of ash (dry weight) in the whole fruit and in the kernel was due to dilution with other materials, e. g., structural carbohydrates and oil laid down

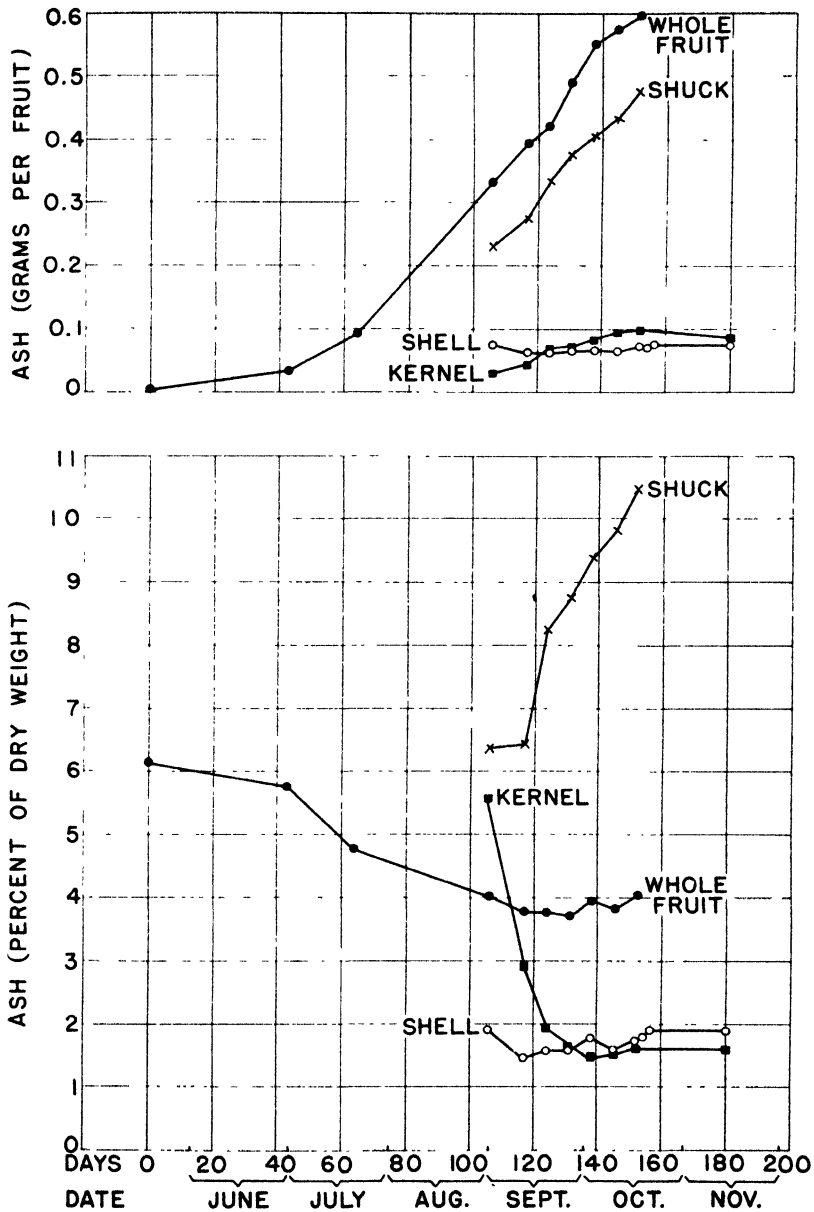


FIGURE 3. Changes in ash content during development of the pecan nut (Burkett variety).

during that time, and not to any loss of mineral constituents. The mineral constituents contained in the kernel showed a gradual increase as filling progressed. All these changes are illustrated in figures 3 and 4.

TOTAL NITROGEN

Figures 5 and 6 show that the nitrogen content of the pecan fruit increase throughout the growing period, although the concentration of nitrogen on a dry-weight basis decreased until filling had begun.

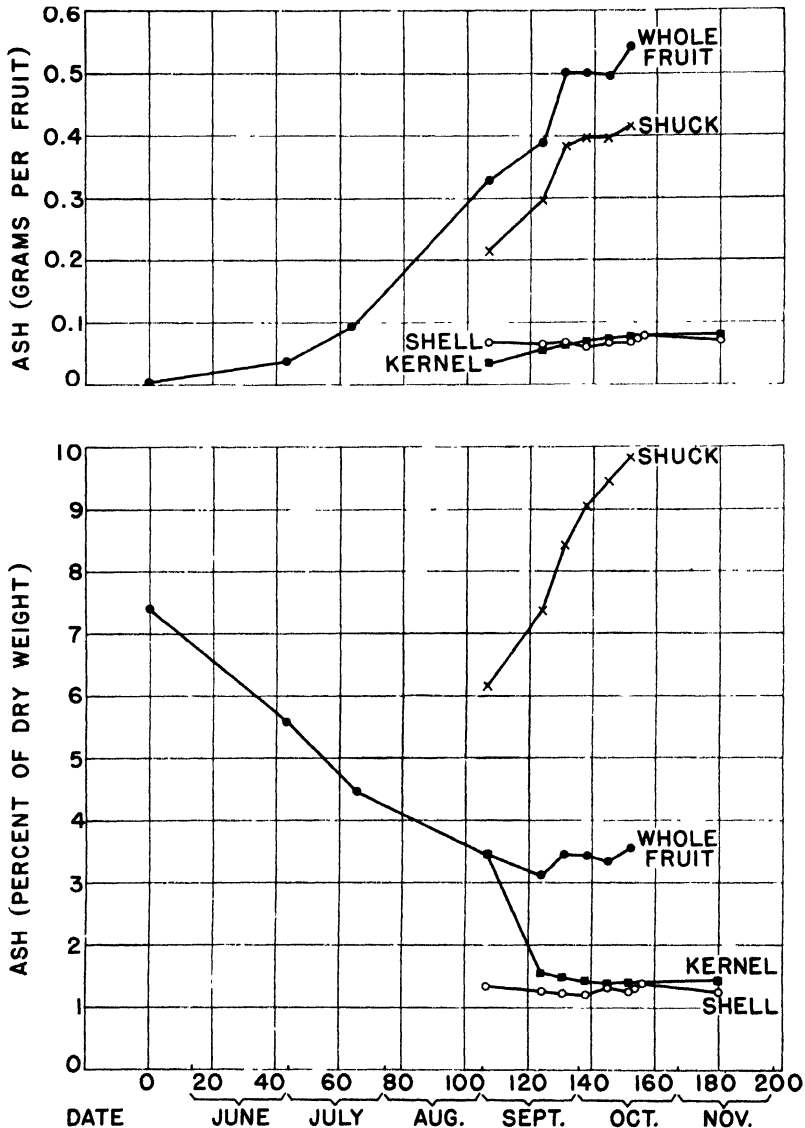


FIGURE 4.—Changes in ash content during development of the pecan nut (Stuart variety).

During the filling process, the nitrogen content of shucks and shells remained practically constant and the increase of nitrogen in the whole fruit was due almost entirely to the deposition of kernel protein. The latter process was very nearly completed during September.

OIL

Figures 7 and 8 show the course of oil formation in the pecan kernel. On September 1 oil synthesis had barely begun, but during the next 30 days 75 to 80 percent of the final oil content of the kernel was laid down. By September 19 the oil in the kernels had reached a concentration of 70 percent on a dry-weight basis, after which time there was a gradual increase until harvest, when the Burkett variety contained about 74 percent of oil and the Stuart variety about 76 percent, on a dry-weight basis. On a green-weight basis the concentration of oil

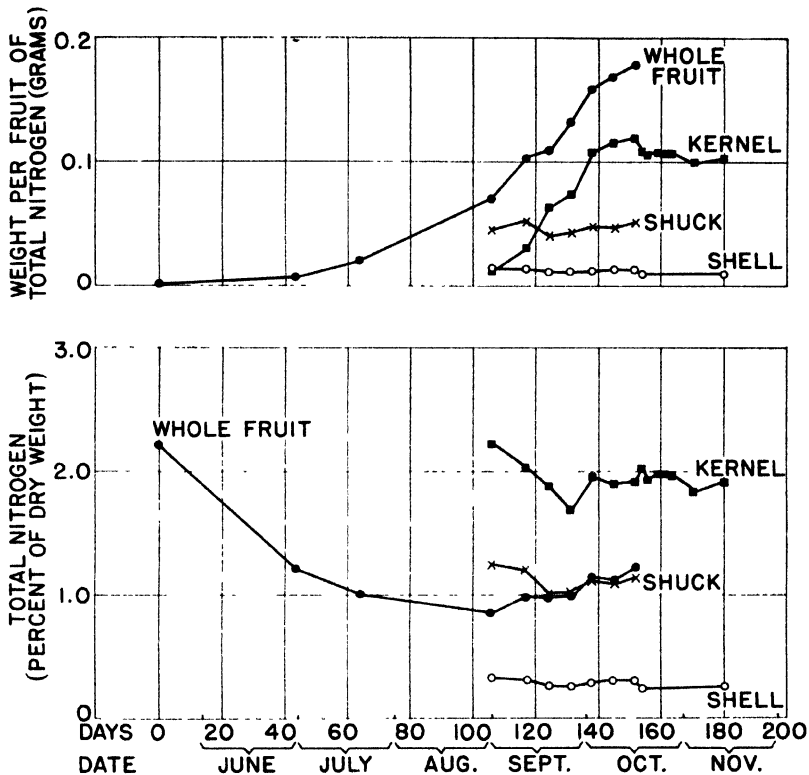


FIGURE 5—Changes in total nitrogen content during development of the pecan nut (Burkett variety).

in the kernels at harvest was near 50 percent in the Burkett and 57 percent in the Stuart, and it increased rapidly for a few days following harvest. However, this increase was due entirely to the loss of moisture from the nuts, since there was no significant change in the actual amount of oil present in the kernel or in the concentration of oil on a dry-weight basis.

The actual amount of oil per nut increased from the time filling began until harvest and remained relatively constant thereafter. The sudden drop at harvest time in the case of the Burkett variety is almost certainly a result of the necessity of changing to a new group of trees for after-harvest samples.

The values for free fatty acids in the oil are not given, because they were insignificantly low and showed no definite trend. The oil from the earliest kernel sample was highest, with a value of 0.7 percent, but the small amount of oil available made the error of determination very great. All subsequent values were less than 0.25 percent. In general, the data indicate no real tendency toward accumulation of free fatty acids prior to glyceride formation.

SUGARS

In considering the results of sugar analyses it seems adequate to discuss only total sugar values, since for any one tissue practically all

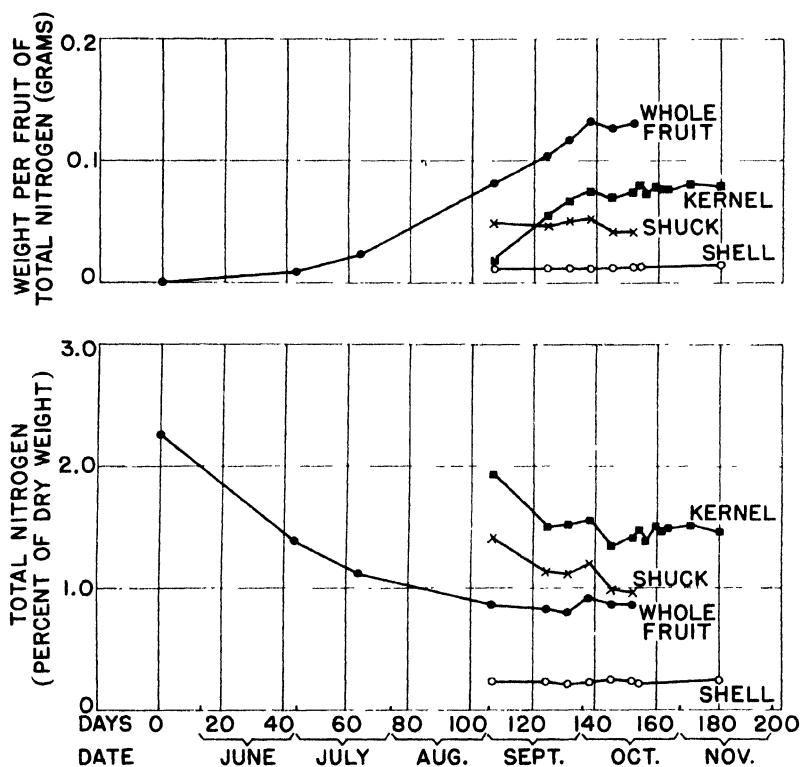


FIGURE 6 -- Changes in total nitrogen content during development of the pecan nut (Stuart variety)

of the sugar was found either in the reducing or in the nonreducing form and no definite shift from one to the other was observed. Except in the very early watery stage the kernel contained almost exclusively nonreducing sugar, all indications being that it was sucrose. In other tissues reducing sugar was the predominant form.

Figures 9 and 10 show the changes in sugar values during the whole period of fruit formation. The sugar concentration in the whole fruit on a dry-weight basis rose to a sharp maximum near the middle of July, and thereafter decreased steadily until early October, when it remained relatively constant. On the weight-per-fruit basis, however, the maximum sugar content was not reached until the first part

of September. During the period of rapid production of oil and protein in the kernel there was a significant drop in the sugar content of the whole fruit.

In the shucks, both the sugar content per shuck and the sugar concentration on a dry-weight basis rose from September 1 until the formation of oil and protein were well under way, then dropped sharply.

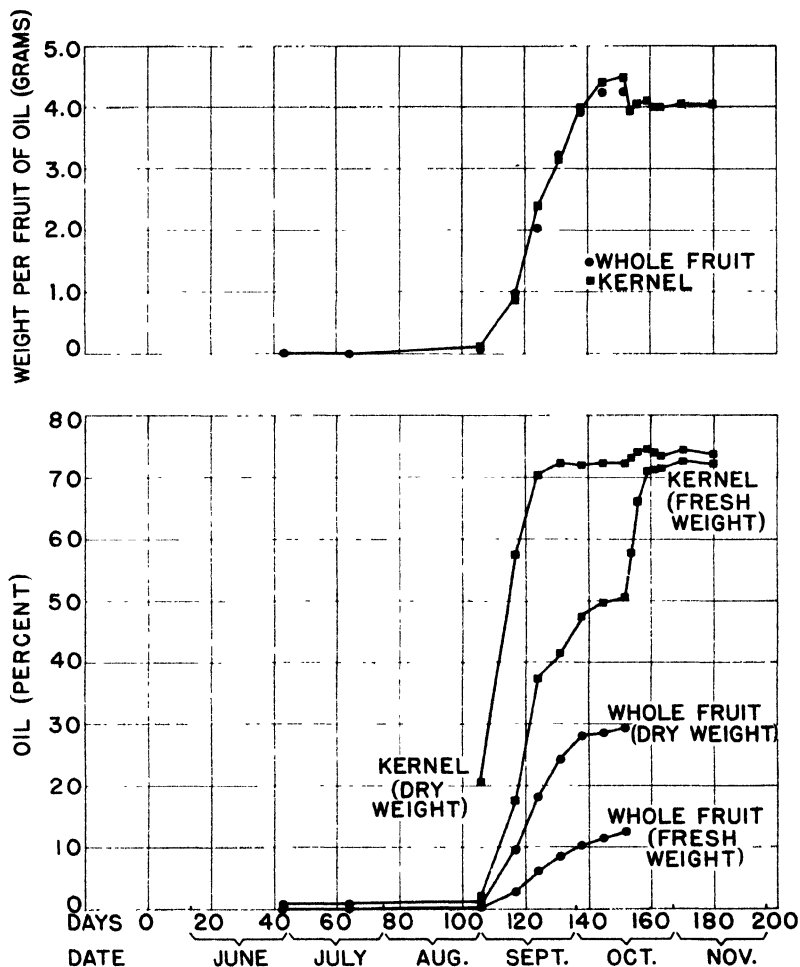


FIGURE 7 Changes in oil content during development of the pecan nut (Burkett variety).

The relatively high sugar content of the early shell samples decreased rapidly during the first 2 weeks of September, remaining at a very low level thereafter.

Only one sample of the kernel in the watery stage was obtained, and it contained a very high concentration of sugar on a dry-weight basis, although the actual amount of sugar per nut was relatively small. The sugar concentration on a dry-weight basis as well as the amount per kernel dropped to a very low level at the time oil synthesis began and

remained low until most of the oil had been formed. Then there was a very rapid accumulation of nonreducing sugar until harvest. Of special interest is the fact that more than 50 percent of the sugar content of the mature kernel was laid down in the 2 weeks immediately preceding harvest. No definite influx of sugar into the whole fruit from the trees was observed during this period, but there was a drop

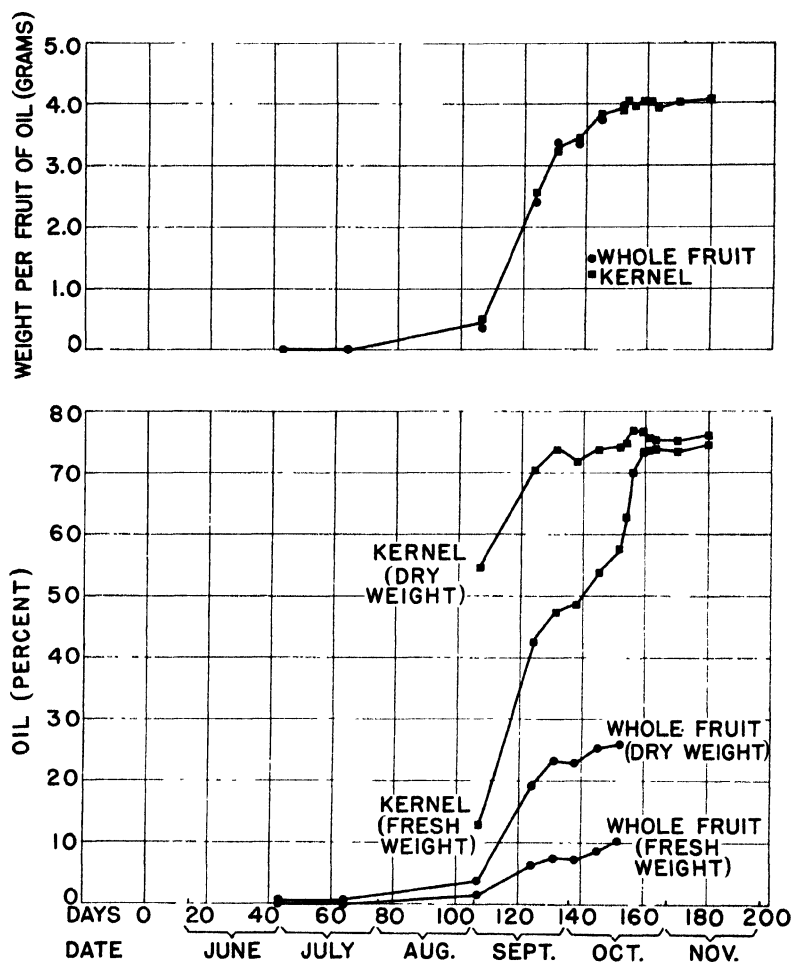


FIGURE 8.—Changes in oil content during development of the pecan nut (Stuart variety).

in the sugar content of the shuck corresponding roughly in amount to the increase in the kernel.

Figures 11 and 12 show the changes in the ratio of total sugar to moisture content. Assuming that all the sugar was dissolved in the portion of the water that was removed by the procedure used for moisture determinations, this should give an approximation of the concentration of sugar solution in the tissues. Calculated in this manner, the sugar concentrations show surprisingly small variations

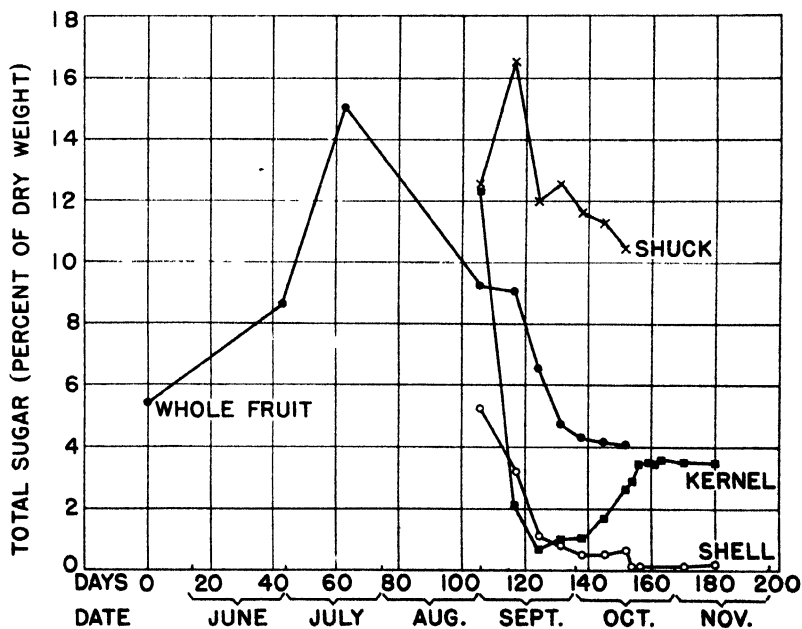
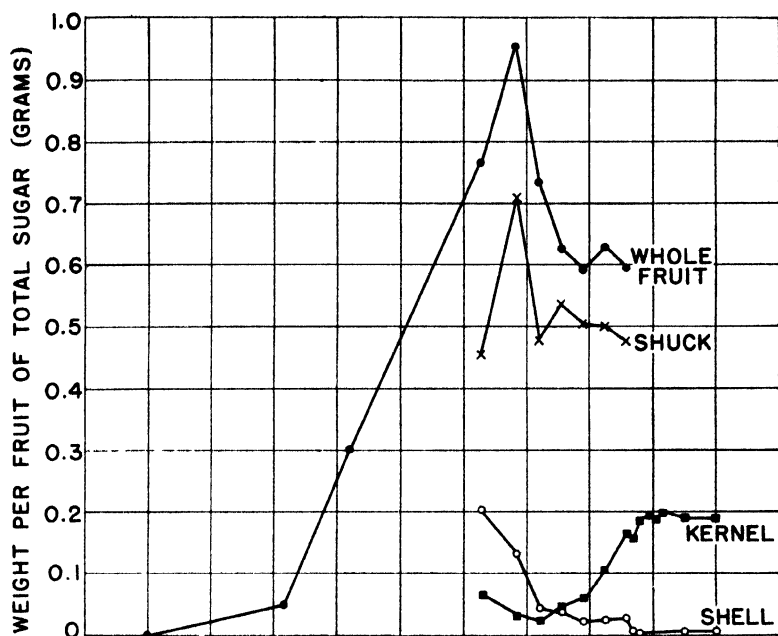


FIGURE 9.—Changes in total sugar content during development of the pecan nut (Burkett variety).

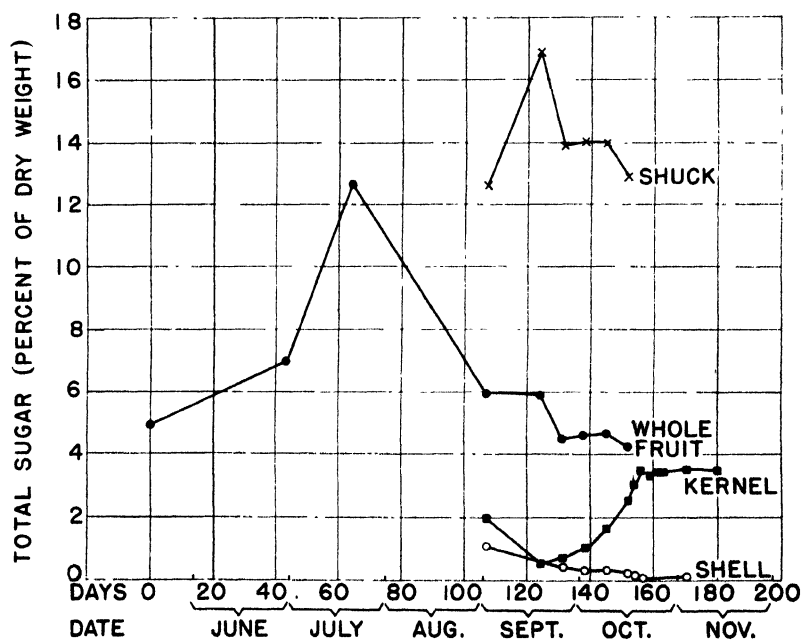
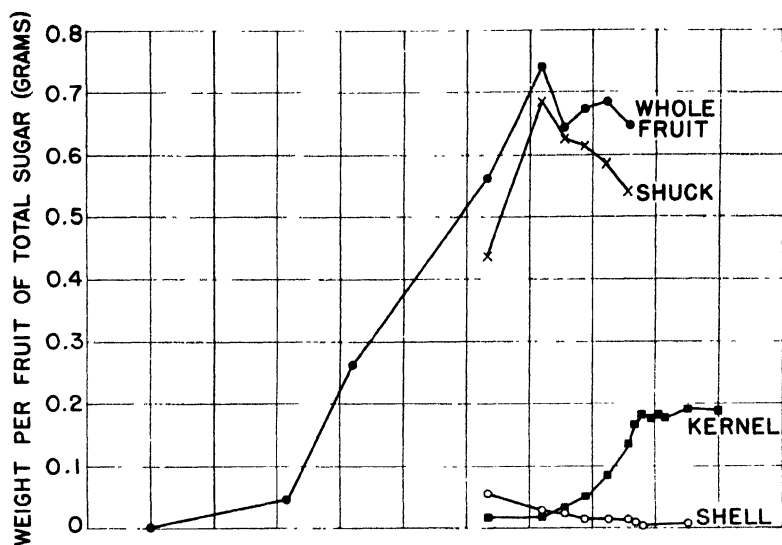


FIGURE 10.—Changes in total sugar content during development of the pecan nut (Stuart variety).

except for the kernel tissues. Although the major portion of the increase in the ratio for kernel tissues occurred as a result of dehydration after harvest, the curve had started definitely upward by the

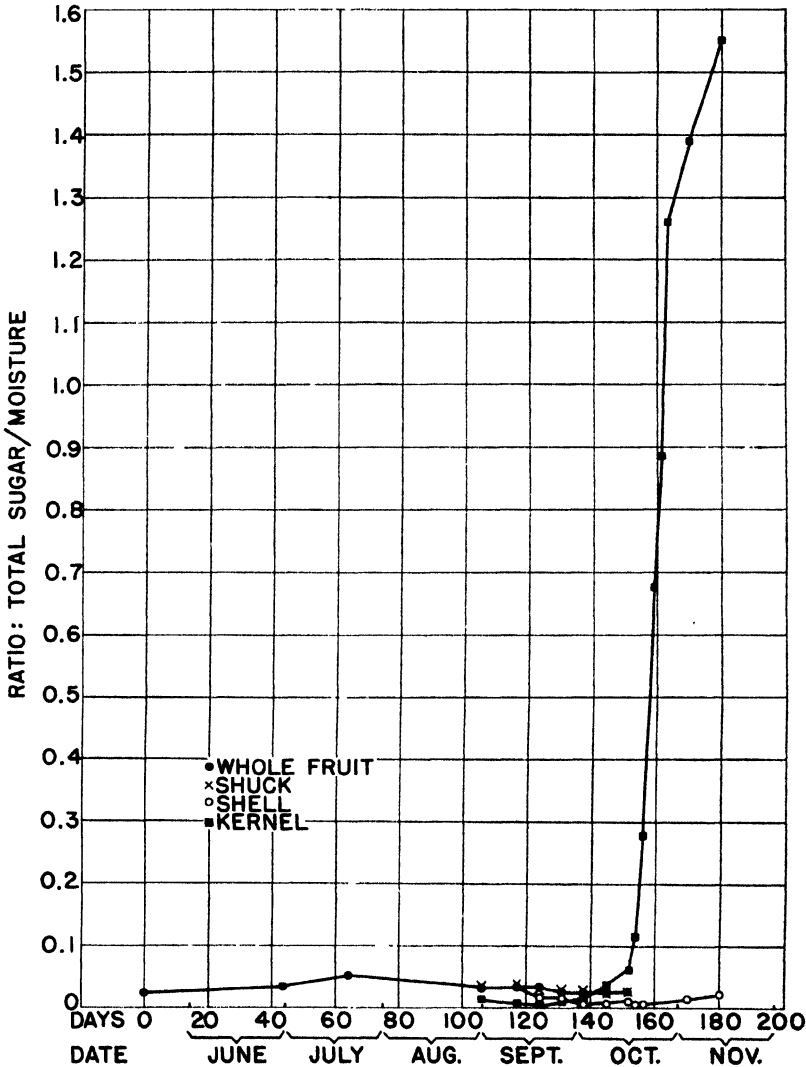


FIGURE 11 —Ratio of total sugar to moisture during the development of the pecan nut (Burkett variety).

middle of September, when syntheses of oil and of protein were at their height.

POLYSACCHARIDES

The changes in acid-hydrolyzable polysaccharides are shown in figures 13 and 14. These substances increased rapidly in the whole

fruit during the summer, when shell and other structural parts were forming, and more slowly after filling of the kernel began. From the time tissue separations were first made, the acid-hydrolyzable polysaccharides of shuck and shell portions showed no important change.

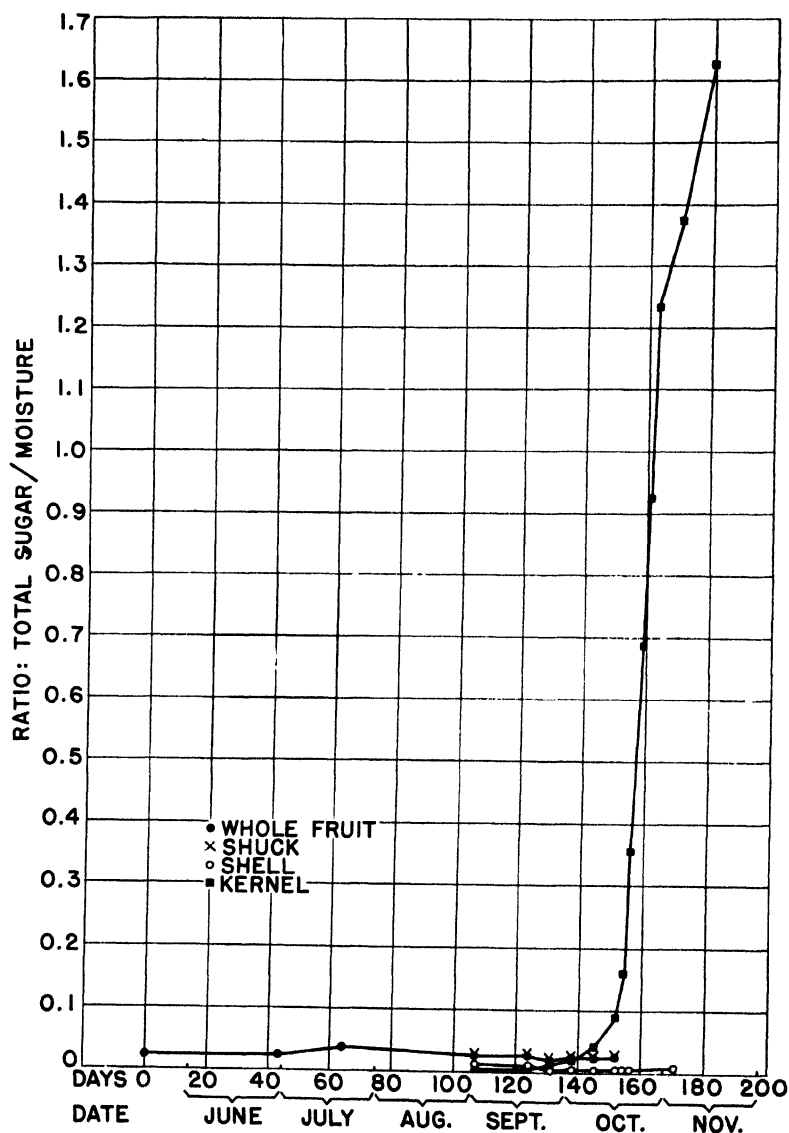


FIGURE 12.—Ratio of total sugar to moisture during the development of the pecan nut (Stuart variety).

Certainly they were not functioning as carbohydrate reserves from which the sucrose so rapidly deposited in the kernel could be derived.

The acid-hydrolyzable polysaccharides in the kernel increased gradually during the filling process. Since these values had to be

calculated as the difference between the whole-fruit data obtained with one lot of 50 nuts and the shuck plus the shell data obtained with a second lot of 50 nuts, any difference in the two lots due to sampling error would show up in the kernel value. This may explain the position of the last point on the Burkett kernel curve.

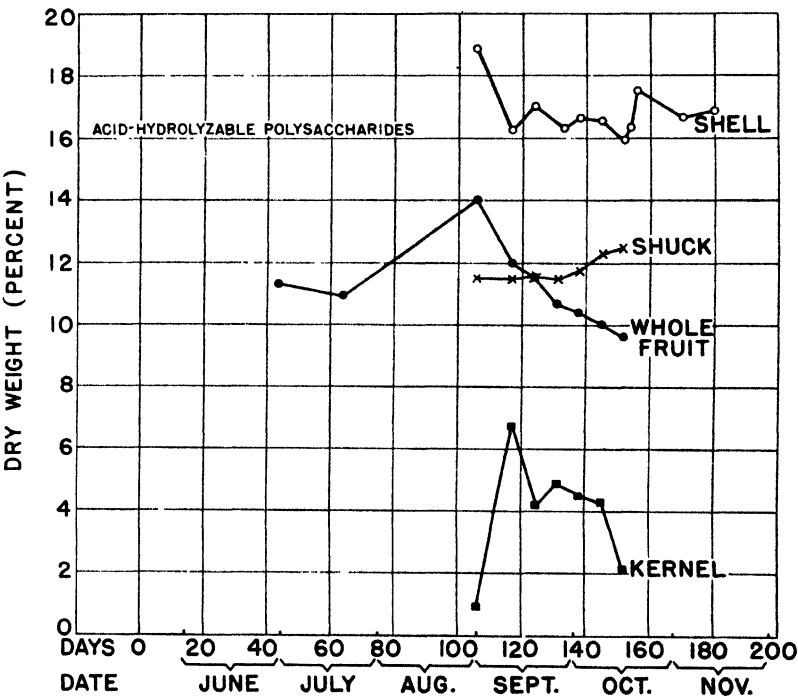
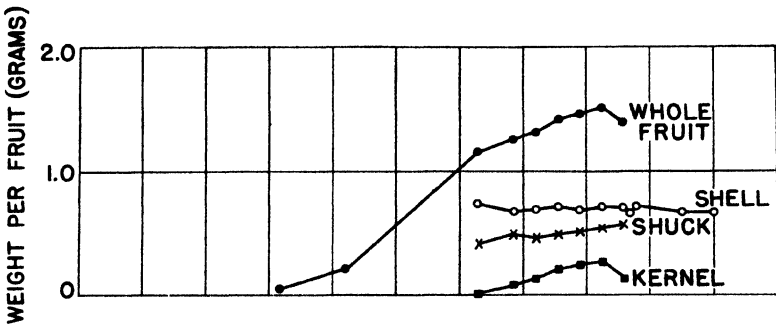


FIGURE 13.—Changes in acid-hydrolyzable polysaccharide content during the development of the pecan nut (Burkett variety).

GENERAL DISCUSSION

For present purposes, the development of the pecan fruit may be divided into two more or less distinct periods. The first comprises the time from blossoming until the kernel begins to fill, i. e., from early May to late August or early September in central Texas. The second

period covers the processes of filling and ripening of the nut, the term "filling" referring to building up of actual dry-matter content of the kernel.

Because of the large number of fruits necessary for an adequate sample, the number of samplings during the first period was necessarily limited and separation into various parts was impracticable. The data obtained show that on a weight-per-fruit basis large increases in all

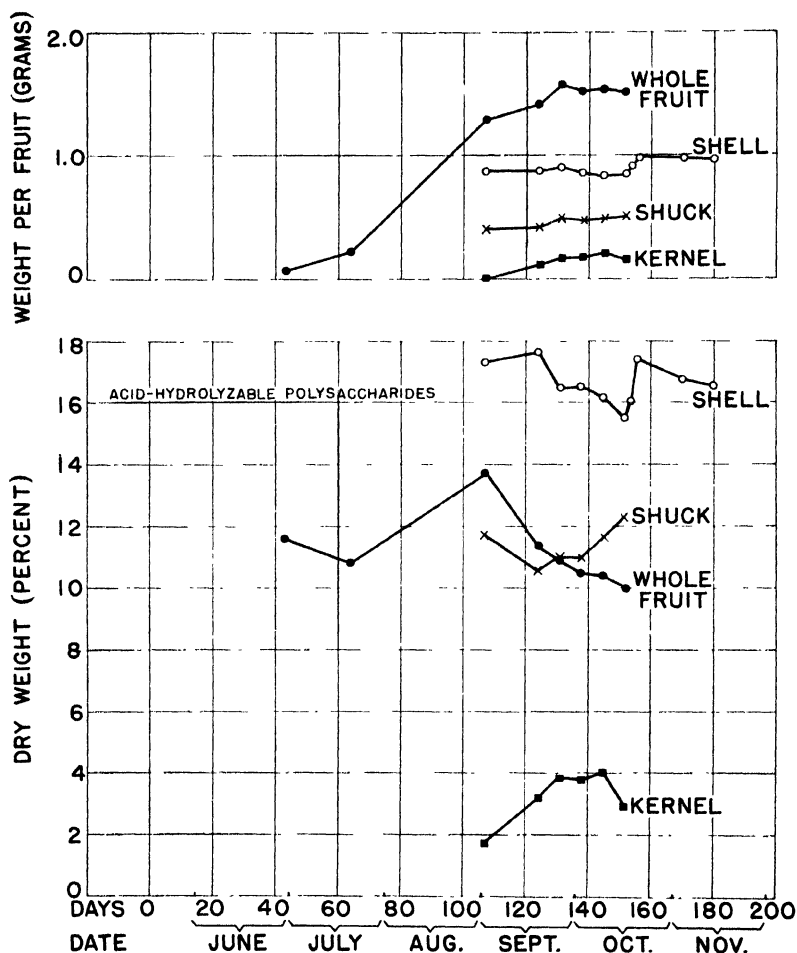


FIGURE 14—Changes in acid-hydrolyzable polysaccharide content during the development of the pecan nut (Stuart variety)

the determined constituents except oil occurred during this time. In the case of total nitrogen and mineral constituents, however, the increase was not so rapid as that of structural materials in general, so that the percentage of nitrogen and ash on a dry-weight basis showed a fairly regular decrease. The sharp maximum in the concentration of total sugar occurring in July may be related to the observations of Woodroof and Woodroof (31, 33, 34, 35) that the fertilized egg begins

to undergo division at about that time. The rise in percentage of acid-hydrolyzable polysaccharides during August was very probably the result of the later stages of shell formation.

After the beginning of the filling process about September 1, the amount of ash in the shell and of total nitrogen and acid-hydrolyzable polysaccharides in both shell and shuck remained practically constant. On the other hand, mineral constituents, protein, oil, and acid-hydrolyzable polysaccharides began to accumulate in the kernel at a rapid rate, and deposition of these substances continued until harvest. The major portion of each of these constituents, however, accumulated in the kernel as early as October 1.

These results agree in every essential with the data given by Woodroof and Woodroof (33). However, their conclusions that the decrease in percentage of nitrogen-free extract was due to a decrease in soluble carbohydrates and that the increase in percentage of oil was due to transformation of carbohydrate to oil appear to the writers not to be justified by the data presented, since the actual amount of nitrogen-free extract in the kernel in grams did not decrease but instead more than doubled during the period in question. Moreover, the recorded decreases in percentage of ash, protein, and fiber, as well as nitrogen-free extract, are largely the results of dilution with oil formed during the period, and represent actual increases in the amounts of these substances present in the kernel. The introduction into the kernel of 60 to 70 percent of its final weight as oil during the course of 4 to 6 weeks makes the drawing of conclusions from percentage composition data alone unusually hazardous.

The idea apparently becoming prevalent in the literature that the kernel of the pecan first fills with carbohydrate material which rapidly changes over to oil during the ripening process is entirely without a quantitative basis and is not borne out by the present study. The decrease in sugar content of the whole fruit during the period of oil synthesis, which is of greater magnitude than the decrease of sugar in any part, could account for not over 5 percent of the final oil. The highest total amount of sugar found at any time could not make more than 12 percent of the normal oil content. The acid-hydrolyzable polysaccharide fraction, which should be next in point of availability, shows no significant decrease in any part, and the whole amount in the kernel itself could not possibly make 3 percent of the final oil content. As a matter of fact, a simple calculation shows that 1 g of glucose contains only sufficient carbon to make 0.517 g of triolein, which, according to Boone (4) and Jamieson and Gertler (20), constitutes about 80 percent of pecan oil. If it be assumed that the energy difference between carbohydrate and fat also comes from combustion of glucose, as is probably the case, then the figure becomes only 0.368 g triolein from each gram of glucose as a maximum limit. By the first of September, when oil production was just beginning, the average dry weight of the whole fruit was 8.27 g for the Burkett variety and 9.45 g for the Stuart variety. If this entire dry weight had been pure glucose, and if it were completely changed into oil during ripening, it could not possibly have given more than 9.45×0.517 or 4.88 g of oil, and the more probable maximum would have been 9.45×0.368 or 3.48 g. Actually, both varieties produced very nearly 4 g of oil per fruit after September 1 and during the same period showed an increase in average dry weight of approximately 6 g.

Both the present data and simple calculations of the relationships involved leave no doubt that practically all of the oil content of the pecan kernel is formed from materials brought into the fruit from other parts of the tree at the time of oil formation. Whether the source of this material is reserve carbohydrates in twigs, trunk, or roots, or whether it is photosynthetic activity during the filling period, remains to be determined. There is no reason to suspect that a similar situation does not exist in the case of most of the protein content of the kernel. At any rate the nitrogen portion of it is definitely shown to be brought into the fruit from the tree at the time of filling.

It was interesting to note that the first samples in which dehiscence between the shuck and the shell was apparent (Oct. 3 for the Burkett variety and Sept. 26 for the Stuart variety) came at points on the curves where the accumulation of the protein and oil began to slow down. These are also the points where the rapid rise in sugar content of the kernel started.

The very marked increase in ash retained in the shuck as compared with that found in the kernel during the filling period may be due merely to an accidental screening out of mineral constituents by the shuck from the relatively large volume of plant sap which must pass through it on the way to the kernel. But whether accidental or not, such a large increase in mineral salts might conceivably have effects on the colloidal state of shuck tissues sufficient to suggest a causative mechanism for the dehiscence between shuck and shell and along the parenchymatous rays of the shuck, which occurs at maturity. Whether or not the latter phenomena have any causal relationship to the filling process, so that studies with a view toward controlling them would be of practical importance, remains to be determined.

The fact that the nonreducing sugar content of the pecan kernel was built up rapidly only after formation of the other constituents had almost stopped indicates that it is a storage form coming from the sugar entering the kernel in excess of that needed for synthesis of oil, protein, etc. Since no significant influx of sugar from the tree into the fruit was observed during the 2 weeks preceding harvest, and since there was a corresponding drop in sugar content of the shucks, it seems probable that most of the sugar content of the mature kernel was the result of translocation from the shuck during the later stages of maturity.

Since the sugar content of the pecan contributes to its quality and flavor, the foregoing results have practical significance in showing that harvesting too early is detrimental, and that possibly, from the quality standpoint, harvesting later than usual might be desirable. It is hoped that this point can be checked when the next season's crop becomes available. Evidence was not obtained to show whether or not a similar sugar increase would occur in nuts separated from the shuck.

It must be considered also that, while the greater amount of oil was synthesized in the kernel several weeks before ordinary harvest time, the amount of oil formed in the kernels during the later period is of enough importance to make it inadvisable to harvest before the nuts are ready to fall from the shucks. A similar situation holds, perhaps to a lesser extent, for the protein content of the nut.

The relatively short time during which the greater amounts of oil and protein were synthesized indicates a critical period in the filling

of nuts. It is probable that proper cultural practices immediately preceding and during this period may aid considerably in filling out a pecan crop when existing natural conditions happen to be unfavorable.

SUMMARY AND CONCLUSIONS

A thorough analytical study has been made of the physiological development of the pecan fruit and several of its parts from the time of flowering until the fruit is mature.

The two varieties used, Stuart, of eastern origin, and Burkett, of western origin, but both grown in central Texas, gave very nearly identical results.

A first period of growth from blossoming until late August or early September was characterized chiefly by formation of structural elements of the shuck and the shell. During the second period filling of the kernel was of major importance.

Most of the oil, protein, mineral, and acid-hydrolyzable polysaccharide content of the kernel developed during September. It is shown that practically all of the material from which oil is formed must be brought in from outside the fruit during the filling period. The same is probably true of protein also.

Very nearly the whole sugar content of the kernel, which is almost exclusively in a nonreducing form, appeared during the first half of October.

The data indicate that much of the sugar in the mature kernel may be a result of translocation from the shuck during the later stages of maturity.

Harvesting pecans before they are ready to fall from the shucks would seem to be inadvisable from the viewpoint of the quality of the nut.

A critical period in the filling of pecan nuts under central Texas conditions seems to occur during September.

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A TETRAPLOID HYBRID OF MAIZE AND PERENNIAL TEOSINTE¹

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INTRODUCTION

In a progeny of seven hybrid plants that resulted from pollinating waxy maize (*Zea mays* L.) with perennial teosinte (*Euchlaena perennis* Hitchc.) there appeared one plant with 40 chromosomes instead of the usual 30 chromosomes. The character of the plant and the behavior of its progeny compel the belief that this plant possessed two sets of maize chromosomes together with the two sets normal to perennial teosinte. This paper is a discussion of the contribution made by this plant and its progeny to an understanding of the synaptic attractions of homologous chromosomes in maize-teosinte hybrids.

Annual teosinte (*Euchlaena mexicana* Schrad.) resembles maize in having 10 pairs of chromosomes. That each of the 10 teosinte chromosomes has its homologue in maize seems certain. The two forms are completely interfertile, and in F_1 pollen mother cells meiosis is normal. Perennial teosinte (*E. perennis*) has 20 pairs of chromosomes. It seems clear that perennial teosinte is tetraploid in nature, having two sets of 10 pairs of chromosomes homologous to those of the annual teosinte and maize. This follows from the fact that genes located in a number of the maize chromosomes have been shown to have two allelomorphs in perennial teosinte (2).²

Randolph's (8) experiments give even more convincing evidence. From X-rayed material of annual teosinte he obtained a tetraploid that is practically indistinguishable from perennial teosinte. The F_1 of crosses between perennial teosinte and maize are then in the nature of triploids, although it is certain that the maize and teosinte homologues are not exact duplicates (1), and it seems not improbable that there has been some differentiation of the duplicated chromosomes in *Euchlaena perennis*.

The somatic cells of an F_1 triploid plant have 3 more or less homologous chromosomes corresponding to each of the 10 haploid chromosomes of maize. At meiosis the normal procedure is for 2 of the 3 chromosomes to synapse and separate into different cells, the third member going at random to one or the other of these two cells.

Information regarding the similarity and differences of the duplicate chromosomes in perennial teosinte and the corresponding homologue in maize may be gained from the mating of chromosomes in the formation of gametes when all three homologues are present, i. e., the two teosinte chromosomes may pair with each other or 1 of them may pair with the maize homologue.

If the duplicate chromosomes in the perennial teosinte were exact homologues and differed from those of maize the two *Euchlaena perennis* chromosomes would be expected always to pair, leaving the

¹ Received for publication Oct. 10, 1934; issued April 1935.

² Reference is made by number (italic) to Literature Cited, p. 133.

maize homologue to be distributed at random. On the other hand, if all three homologues were similar or had equal synaptic attraction, random pairing would be expected.

Present knowledge of the morphological characters of the chromosomes of perennial teosinte is too limited to identify the synaptic mates cytologically, but an indirect method of determining the nature of the pairings in F_1 hybrids is afforded by introducing with maize the waxy gene of the ninth chromosome and then observing the ratios of pure recessive, pure dominant, and heterozygous gametes derived from such a cross.

The gene for waxy endosperm (*wx*) is especially serviceable for measuring synaptic attractions because this gene is expressed in the pollen, thus making it possible to determine with any desired degree of accuracy the percentage of recessive male gametes. The procedure was to collect anthers about 1 day before pollen was shed, crush the pollen into a solution of iodine, and classify approximately 500 grains.

In the usual crosses of perennial teosinte and maize which produce a triploid F_1 the situation is complicated by the partial elimination of gametes with aneuploid chromosome numbers (?). In the pollen of a triploid F_1 there are many sterile grains, and these have a low number of chromosomes, thus reducing the percentage of waxy grains in the surviving pollen.

The fortunate occurrence of the tetraploid F_1 plant in which there were two sets of maize chromosomes and little or no sterile pollen affords an opportunity to determine the synaptic attractions of chromosome 9 in a very simple manner.

MEASURES OF AUTOSYNDESIS³

The two homologues of chromosome 9 contributed by the *Euchlaena perennis* parent may be designated X_1 and X_2 , and the two from maize x_3 and x_4 .

At meiosis the four homologues of chromosome 9 may pair in three ways: (1) X_1 with X_2 , and x_3 with x_4 ; (2) X_1 with x_3 , and X_2 with x_4 ; (3) X_1 with x_4 , and X_2 with x_3 . The first is autosyndetic and the second and third are allosyndetic. Since paired chromosomes pass to different gametes the four kinds of gametes formed following the first way of pairing will be $X_1 x_3$, $X_2 x_4$, $X_1 x_4$, and $X_2 x_3$, all genetically similar; i. e., there will be four Xx gametes. The gametes following the second way of pairing will be $X_1 x_2$, $x_3 x_4$, $X_1 x_4$, and $X_2 x_3$, and those from the third will be $X_1 x_2$, $x_3 x_4$, $X_1 x_3$, and $X_2 x_4$. The gametes following the second and third ways of pairing are genetically similar and the combined result of the allosyndetic pairings will be 2 XX , 2 xx , and 4 Xx . Thus, one-fourth of the gametes following allosyndesis will be recessive, whereas all of those following autosyndesis will contain a dominant gene. With equal synaptic attraction—i. e., random pairing—two-thirds of all the gametes will have followed allosyndesis and one-third autosyndesis. Accordingly, one-fourth of two-thirds, or one-sixth of the total gametes will be recessive.

With an increase in autosyndesis the percentage of recessive gametes decreases until with complete autosyndesis there will be no recessive

³ Both the terms "autosyndesis" and "autosynapsis" have been used to designate synapsis inter se of like chromosomes. It would seem that the older term "synapsis" might have served in the new connection and syndesis is used in this paper not because it is thought more appropriate but because it seems to conform with current usage. The term "syndesis" was proposed by Häcker (5, 6).

gametes. With an increase of allosyndetic pairings the percentage of recessive gametes increases until with complete allosyndesis 25 percent of the gametes will be recessive. Where there is no differential elimination of gametes, therefore, the intensity of autosyndetic attraction is indicated by the extent to which the percentage of recessive gametes departs from the 16.7 percent of random pairing.

The percentage of recessive gametes, however, is unsatisfactory as a means of comparing syndetic attractions. The distribution of percentages above and below the 16.7 is not symmetrical, and the increase of autosyndetic pairing indicated by a given minus departure from 16.7 percent does not equal the increase in allosyndetic pairings indicated by a plus departure of the same amount. As an example, if autosyndesis is increased from random pairing to such an extent that the resulting autosyndetic gametes are doubled in number, the percentage of recessive gametes will be reduced from 16.7 to 12.5, a difference of 4.2 percent. If, however, allosyndesis is doubled, the recessive gametes will be 20 percent, a difference of only 3.3 percent.

This skew distribution of percentages may be transformed to a scale running from -1 to 1 , in which -1 =complete allosyndesis, 0 =random pairing, and 1 =complete autosyndesis. Values on this scale may be termed the "coefficient of autosyndesis", and equal values above and below zero will represent equal percentage changes in allosyndetic and autosyndetic pairings. This coefficient of autosyndesis, which will be called t , is derived from the ratio of recessive gametes to all gametes, as follows:

$$t = \frac{1-6x}{1-2x}$$

where x = the ratio of recessive gametes.⁴

Since x is the only variable, the significance of t should equal the significance of the departure of x from 16.7 percent or

$$\sigma_t = \frac{t\sigma_x}{16.7-x}$$

DESCRIPTION OF TETRAPLOID HYBRID

The F_1 hybrid that was found to have 40 chromosomes attracted attention while growing in the field because it was more cornlike than its sister F_1 plants. It had a major culm, and the minor culms were much reduced in size. The usual triploid F_1 shows very little differentiation of a major culm, and the few to many culms that develop the first year are of approximately equal size. Almost all the ears of the 40-chromosome F_1 had 2 rows of double alicoles, an occasional terminal ear having 4 rows of double alicoles. Normal F_1 hybrids usually have ears with 2 rows of single alicoles and only occasionally an ear

⁴ If there is no elimination of aneuploid gametes this formula is applicable to triploid as well as to tetraploid hybrids. Where the mean chromosome number of the gametes is other than one-half the somatic number of the parent the value of t still may be determined if the mean number of chromosomes in effective gametes is known, as follows:

$$\text{Let } x = \frac{\text{percent waxy pollen}}{100},$$

C = somatic number of chromosomes of parent plant,
 c = mean number of chromosomes in the gametes,

$$r = \frac{C-(c+10)}{c-20};$$

$$\text{then } t = \frac{3r-r}{x-r}.$$

with 4 rows. The 40-chromosome plant showed only 2 percent poorly developed pollen, while normal F_1 plants often have as high as 50 percent defective pollen. The percentage of *wx* pollen was between 4 and 5, a percentage higher than any thus far found in F_1 hybrids between perennial teosinte and waxy maize.

The fact that the F_1 plant had 40 instead of the usual 30 chromosomes, together with the habit of the plant and the more cornlike ears, suggested that it had 2 rather than 1 set of maize chromosomes in its complement. The various phases of the reduction divisions tended to confirm this view, since there was little or no indication of unpaired chromosomes and the distribution of the chromosomes to the daughter cells took place with only infrequent indications of irregularities.

The most natural explanation of the presence of the 2 sets of maize chromosomes is that the maize parent contributed 2 sets of chromosomes or that 1 maize set had doubled after fertilization. These are the explanations suggested by Emerson and Beadle (3) in describing another tetraploid hybrid between perennial teosinte and maize. The description of their plant indicates that in general morphological characters it was very similar to the tetraploid hybrids under discussion.

Chromosome studies of F_2 and F_3 generations continue to show very few irregularities in the reduction phases. In some plants the chromosomes on the first metaphase plates show many tetravalent chromosome groups; whether this is due to the association of 4 teosinte or 4 maize chromosomes or simply to the association of both teosinte and maize chromosomes in a group of 4 is difficult to say, since in a certain proportion of the plants of later generations the 4 homologues will be either all teosinte or all maize. The regular distribution of the chromosomes to the daughter nuclei is not disturbed by the presence of tetravalent groups; it is usual to find anaphase figures that have 20 chromosomes going to each pole, and consequently practically all pollen grains are euploid with 20 chromosomes.

PROGENY OF TETRAPLOID HYBRID (F_1)

The plant with 40 chromosomes produced pollen having 4.6 ± 0.94 ⁵ percent of the grains waxy, giving a coefficient of autosyndesis of 0.80 ± 0.06 .

A plant with the composition $Wx Wx wx wx$ would produce gametes with 0, 1, and 2 Wx genes, and when selfed there should be F_2 individuals with 0, 1, 2, 3, and 4 Wx genes.

If pollen and ovules behave alike the percentage of waxy pollen should make possible a prediction as to relative frequency of F_2 plants with 0, 1, 2, 3, and 4 Wx genes. Where $x = \frac{\text{percentage of waxy pollen}}{100}$ the expected frequencies would be:

Number of Wx genes	Expected ratio	Plant characteristics
0	x^2	Waxy seed
1	$2x(1-2x)$	50 percent waxy pollen
2	$2x^2 + (1-2x)^2$	0-25 percent waxy pollen
3	$2x(1-2x)$?
4	x^2	No waxy pollen

⁵ Errors indicated by a \pm sign are standard, not probable, errors.

Classification should be definite except for the class with three *Wx* genes. In the absence of irregular divisions, plants of this type should have no waxy pollen. Unequal divisions do occur, however, and their observed frequency indicates that about 0.5 percent of the gametes of a *Wx Wx Wx wx* plant would be waxy. Accepting this ratio, the chance of any particular pollen grain being waxy is 0.005, and in samples of 500 grains 92 percent of the samples would have one or more waxy grains and would fall in the low-waxy group. With regard to the upper limit, assuming the same probability of 0.005, about 90 percent of the samples from *Wx Wx Wx wx* plants would have less than 5 waxy grains, and would be listed as having less than 1 percent waxy pollen. For purposes of comparison, therefore, plants with less than 1 percent waxy pollen have been classed as *Wx Wx Wx wx*. There was no evidence of bimodality either in the F_2 or F_3 progenies, but in F_3 progenies from selfed *Wx wx wx wx* F_2 plants where there was no opportunity to produce *Wx Wx Wx wx* plants the lowest percentage was 4.

The tetraploid F_1 plant was self-pollinated and produced 276 seeds, 1 of which was waxy. The expected number of waxy seeds was 0.6.

DISTRIBUTION OF F_2 PLANTS

Fifty-six F_2 plants were grown, and approximately 500 pollen grains of each were classified by staining with iodine. The results are given under the column heading F_2 of table 1.

The 5 plants in the group approximating 50 percent waxy pollen are sharply differentiated from those with smaller percentages and undoubtedly are plants with one *Wx* gene. The one plant with no waxy pollen showed by the subsequent behavior of its progeny that it was homozygous *Wx*. The only uncertainty regarding the genetic composition of the plants is that of separating those with three *Wx* genes from those with two. The number of plants in the several groups is in good agreement with the expected.

DISTRIBUTION OF F_3 PLANTS

Nine F_3 progenies were grown in 1933 from nonwaxy seed of F_2 plants. Two progenies were from selfed plants with 50 percent waxy pollen (*Wx wx wx wx*), 4 were from selfed plants with a low percentage of waxy pollen (*Wx Wx wx wx*), and 3 were from crosses between the two groups (*Wx Wx wx wx* \times *Wx wx wx wx*).

A total of 435 F_3 plants were grown and the pollen grains of each were classified. With one exception the plants, like those of the F_2 , fell into two well-defined groups with respect to percentage of waxy pollen. The single exception was a plant with 31.4 percent of waxy pollen in progeny C_3 . This progeny was the result of selfing an F_2 plant with 51.3 percent of waxy pollen. The aberrant plant had 40 chromosomes and there was no indication of cytological irregularities. Until the descendants of this plant are grown no explanation can be offered for its unexpected percentage of waxy pollen; the plant is omitted from the tabulations.

The results are given in table 1 under the column heading F_3 .

TABLE 1.—Distribution of percentage of waxy pollen in F_2 and F_3 plants

Genetic group and percentage of waxy pollen (class value)	F ₃ plants of indicated parentage									F ₂ plants
	Progenies (upper) and percentages of waxy pollen (lower) of <i>Wx Wx wx wx</i> × self				Progenies (upper) and percentages of waxy pollen (lower) of <i>Wx wx wx wx</i> × self		Progenies (upper) and percentages of waxy pollen (lower) of <i>Wx wx wx wx wx</i> × <i>Wx Wx wx wx</i>			
	C ₂	C ₆	C ₇	C ₉	C ₁	C ₃	C ₄	C ₈	C ₁₀	
	7.9	11.2	4.0	1.5	50.9	51.3	51.3×7.9	4.0×50.9	1.5×50.9	
<i>Wx Wx Wx Wx Wx</i> : 0.....	Number 0	Number 0	Number 0	Number 0	Number 0	Number 0	Number 0	Number 0	Number 0	Number 1
<i>Wx Wx Wx wx</i> : 0.1-0.9.....	3	1	4	2	0	0	1	0	2	2
<i>Wx Wx wx wx</i> : 1.0-1.9.....	5	2	8	1	---	---	---	---	1	4
2.0-2.9.....	6	3	14	7	---	---	---	1	2	8
3.0-3.9.....	15	5	15	9	---	---	---	1	3	7
4.0-4.9.....	9	5	12	13	3	---	---	4	1	8
5.0-5.9.....	0	6	7	6	1	4	3	2	2	6
6.0-6.9.....	1	3	4	4	2	2	3	4	2	2
7.0-7.9.....	2	6	1	0	2	1	1	2	0	5
8.0-8.9.....	1	5	1	1	1	3	4	---	4	2
9.0-9.9.....	4	3	1	2	1	0	3	---	3	3
10.0-10.9.....	3	4	0	---	0	1	0	---	1	1
11.0-11.9.....	---	4	1	---	0	1	0	---	1	1
12.0-12.9.....	---	0	---	---	1	0	4	1	---	---
13.0-13.9.....	---	0	---	---	1	0	3	---	---	---
14.0-14.9.....	---	1	---	---	---	0	---	---	---	---
15.0-15.9.....	---	1	---	---	---	1	---	---	---	---
16.0-16.9.....	---	1	---	---	---	---	---	---	---	1
Plants number.....	46	49	64	43	12	13	21	15	20	48
Mean waxy pollen percent.....	4.7	7.2	4.0	4.5	7.6	8.1	9.4	5.9	6.5	5.2
Observed σ.....	2.7	3.5	2.0	1.8	3.0	2.8	2.9	2.3	3.0	3.1
Expected σ.....	11.1	13.7	10.3	10.8	14.3	14.8	16.1	12.5	13.2	11.7
<i>Wx wx wx wx</i> 44.0.....	.95	1.16	.88	.93	1.19	1.22	1.31	1.06	1.10	.90
45.0.....	---	---	---	---	---	---	---	1	---	---
46.0.....	---	---	---	---	---	---	---	0	1	---
47.0.....	---	---	---	---	---	4	---	2	1	---
48.0.....	---	---	---	---	---	4	2	0	0	---
49.0.....	---	---	1	2	3	8	4	3	1	---
50.0.....	---	4	0	0	9	7	5	1	2	2
51.0.....	---	0	0	3	3	6	2	2	1	1
52.0.....	---	2	1	1	2	5	5	3	---	1
53.0.....	---	0	0	1	3	7	6	1	1	1
54.0.....	---	0	1	---	2	1	1	1	---	---
55.0.....	---	1	1	---	2	1	1	1	---	---
56.0.....	---	0	---	---	2	---	---	---	---	---
57.0.....	---	1	---	---	---	---	1	---	---	---
58.0.....	---	---	---	---	---	---	---	---	---	---
59.0.....	---	---	---	---	---	---	---	---	---	---
60.0.....	---	---	---	---	---	---	1	---	---	---
Plants number.....	0	8	4	7	26	43	28	15	7	5
Mean waxy pollen percent.....	---	52.5	53.0	51.4	52.2	50.9	52.2	5.08	48.8	51.7
Observed σ.....	---	2.7	2.6	1.5	2.2	2.1	2.7	2.9	2.3	1.3
Expected σ.....	---	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2

In some progenies the number of plants in the several genetic groups depart rather widely from the expected, but the totals are in fair agreement.

The number of plants classified as having three Wx genes is low, which may indicate that some of the plants with more than 1 percent of waxy pollen should have been placed in this group.

PERCENTAGE OF WAXY POLLEN IN F_2 AND F_3

The percentage of waxy pollen in individual plants having the composition $Wx\ wx\ wx\ wx$ was a close approximation to the expected 50 percent. The progeny means, ranging from 48.8 to 53.0 percent in some instances, deviated significantly from 50 percent, but the departures are no greater than are commonly observed in maize hybrids. Plants having the composition $Wx\ Wx\ wx\ wx$ ranged from 1.1 to 16.6 percent waxy pollen. Progeny means ranged from 4.0 to 9.4 percent, many of the differences being of undoubted significance. The values are given in table 1.

COEFFICIENT OF AUTOSYNDESIS

The percentage of waxy pollen of any plant with two Wx genes affords a measure of autosynopsis in that plant. Of this type there were, in addition to the F_1 individual, 48 F_2 and 283 F_3 plants. In the F_1 the coefficient of autosynopsis $t=0.80$. The mean value of t in the F_2 was 0.77 ± 0.03 . In the entire population of F_3 progenies the mean value of t was 0.74 ± 0.05 . The slight evidence of a decrease in t is insignificant.

The 283 F_3 plants were in 9 progenies. Two of these progenies were the result of selfing F_2 plants with approximately 50 percent waxy pollen, 4 resulted from selfing F_2 plants with a low percentage of waxy pollen, and 3 were from crosses between plants with a low percentage of waxy pollen and plants with approximately 50 percent waxy pollen.

The mean values of t in these three groups were as follows:

Parents	t	Standard error
50 percent \times self	0.63	0.05
Low percent \times self	.78	.05
50 percent \times low	.65	.08

Again the differences are insignificant, the differences between the extremes being only 2.16 times the standard error of the difference. It should perhaps be noted that the order of the coefficients is that expected if crossing-over, which may have occurred in the F_1 and F_2 , were a factor in reducing autosynopsis.

VARIATION IN COEFFICIENT OF AUTOSYNDESIS

One of the most striking things brought out in table 1 is the greater variability of the percentage of waxy pollen in plants with two Wx genes than in plants with one Wx . The magnitude of this difference is not apparent in the standard deviations of the arrays, the true difference being obscured by the fact that the expected random variation due to errors of sampling decreases as the mean ratio departs from 0.5 or 50 percent. The standard deviations may be made comparable by multiplying each standard deviation by the

factor $\frac{0.5}{\sqrt{pq}}$, where $p = \frac{\text{percent waxy}}{100}$ and $q = 1 - p$. Standard deviations thus corrected to a base of 50 percent are designated σ_{50} in table 1. For the plants with one Wx the mean values are so close to 50 percent that the correction is negligible. A comparison of these corrected values shows the standard deviation of the plants with two Wx genes

to be from 4 to 9 times that of plants in the same progeny with one Wx .

The same difference is apparent if the observed standard deviations are compared with their respective errors. In every progeny the standard deviation of plants with one Wx was a close approximation to the 2.2 percent expected as a result of sampling errors in samples of 500. In the plants with two Wx genes the observed standard deviation is significantly greater than the expected in every progeny.

A general comparison of the variability of plants having one Wx gene with the variability of those having two Wx genes can be made by analyzing the total variance of the entire population of F_3 plants. Snedecor (9) describes the analysis of variance as "a technique for segregating from comparable groups of data the variation traceable to specified sources."

As applied to the present data the analysis of variance combines all the classified pollen grains from F_3 plants in each of the genetic groups into a single population and segregates the total variance into the portions contributed by the several subdivisions. The total variance is $\overline{pq} \overline{N}$, i. e., the $\frac{\text{percent waxy}}{100}$ times the $\frac{\text{percent horny}}{100}$ of the entire population times the total number of pollen grains. A portion of this total variance will be accounted for by the $pq \overline{N}$ of the individual plants, another portion will be due to departures of the percentage of waxy pollen of individual plants from the mean of the progeny in which they occur, still another portion will be due to departure of the progeny means from the mean of the type of cross—i. e., 1 Wx by self, 2 Wx by self, and 1 Wx by 2 Wx —and lastly there will be a portion due to departures of means of the three types of crosses from the general mean. If these several divisions constituted merely random samples from a homogeneous population, the mean square would be essentially the same for all the divisions. The values of mean-square differences required for significance are given in a table at the end of Snedecor's work (9) and by Fisher (4).

All sums of squares have been divided by 500, which, without changing the relative values, reduces the mean squares to the basis of the variance of means of plants in the progeny.

The analysis of variance of all the F_3 plants of the composition $Wx wx wx wx$ and $Wx Wx wx wx$ is shown in table 2.

TABLE 2.—Analysis of variance of all F_3 plants of the composition $Wx wx wx wx$ and $Wx Wx wx wx$

Genetic composition and source of variation	Degrees of freedom	Sum of squares	Mean square
$Wx wx wx wx$:			
Types of parental cross	2	15	7.5
Progenies in type	5	135	27.0
Plants in progeny	131	729	5.6
Pollen in plant	69,361	346,457	5.0
$Wx Wx wx wx$:			
Among means of types of parental cross	2	366	183
Progenies in type	6	452	75
Plants in progeny	274	1,937	7.1
Pollen in plant	141,217	138,311	.98

In the plants with one *Wx* the mean-square values are practically alike except the value for the means of progenies. This value is significantly larger than the mean square of plants in the progeny or of pollen in individual plants. The probability is somewhat less than 0.01 in both cases.

In plants with two *Wx* genes the variation among types of the parental cross is the only class that does not show a significant difference in comparison with other sources of variation.

The point of interest in connection with variation in autosynthesis is that in the plants with one *Wx* gene (where there is no opportunity for autosyndetic attractions to operate) variation of plants from the mean of the progeny is no greater than that expected from sampling errors, whereas in the plants with two *Wx* genes the variance of plants from the mean of the progeny is over seven times the expected.

The variation in the percentage of waxy pollen might result from a differential survival of the waxy pollen before the pollen was classified, but if so the same differentiation would be expected in the plants with one *Wx* gene and it seems more reasonable to associate the variation with variation in autosynthesis.

There is evidence that the tendency toward autosynthesis is inherited. The four F_3 progenies of the 2-*Wx*-by-self type of cross have a parent-offspring correlation of 0.82 between the mean percentage of waxy pollen of the parent plants and the mean percentage of the progenies. As this value is based on but four pairs it is not significant, but since the values for the offspring are the means of 50 or more plants the standard error must be somewhat less than the indicated value of 0.18.

SEED CLASSES

The F_3 plants that provided the material for estimating the ratio of autosynthesis to allosynthesis in the formation of pollen were pollinated with pollen from pure waxy plants. The seed thus produced provided material for comparing the behavior of the ovules with that of the pollen. The number of seeds obtained from individual plants was too small—few plants produced more than 100 seeds—to justify the kind of analysis used with the pollen. The numbers are sufficient however, to supplement some of the conclusions based on the pollen counts. About 270 F_3 plants of which the pollen was counted produced seed. In no plant was there a disagreement regarding the indicated genetic constitution. Classified on the percentage of waxy seed the plants fell into two groups, one approximating 50 percent and the other having a low percentage; these groups corresponded to those based on the pollen classification.

Rather critical evidence that the variation in the percentage of waxy in the low-percentage group is caused by variation in autosynthesis is provided by the correlations between the percentage of waxy pollen and the percentage of waxy ovules of the same plant. If variation in the percentage of waxy is the result of differences in the coefficient of autosynthesis, variations in syndetic attraction might be expected to affect pollen and ovules alike and there should be a positive correlation between the percentage of waxy pollen and the percentage of waxy ovules in the low-percentage group. In the 50-percent group, where one-half of all pairings must be between mates and

one-half between nonmates, the variations in percentage of waxy are assumed to be due to sampling errors and there should be no correlation between pollen and ovules. In 64 plants in the low-percentage group with a total of 7,106 seeds, the total correlation was 0.36; that between the means of the 8 progenies, 0.72; and that within the progenies, 0.27. The total correlation is undoubtedly significant ($P=0.0016$). The interprogeny and intraprogeny coefficients have each a probability of 0.02. Although the numbers are small the interprogeny correlation indicates that the tendency to autosyndesis is inherited.

In the 50-percent group there were 93 plants with 8,228 seeds. The correlation coefficients (total, -0.065 ; between means, 0.015 ; within progenies, -0.071) were as near zero as could be expected.

SUMMARY

The occurrence of a tetraploid plant among F_1 hybrids between perennial teosinte (*Euchlaena perennis* Hitchc.) and waxy maize (*Zea mays* L.) is reported.

The tetraploid plant was more maizelike in its morphological characters than the usual triploid hybrids of the same parentage. The 40 chromosomes of the somatic tissue of the tetraploid plant are believed to be made up of 20 from the perennial teosinte, the normal haploid number, and 2 sets of maize chromosomes.

Meiosis was regular and there were very few sterile pollen grains.

At meiosis the pairing may be of two types: (1) The teosinte chromosomes may pair with each other and the maize chromosomes do likewise, which is autosyndesis, or (2) the teosinte and maize homologues may pair, which is allosyndesis. The relative frequency of these two types of pairing may be measured by the percentage of gametes carrying the recessive gene *wx* introduced with the maize parent.

A formula is given for calculating the coefficient of autosyndesis, which transforms the skew distribution of percentages into a symmetrical distribution. With this measure, called t , complete autosyndesis is represented by 1, random pairing by 0, and complete allosyndesis by -1 .

The F_2 and F_3 progenies produced plants with 0, 1, 2, 3, or 4 dominant *Wx* genes in approximately the expected ratios, indicating a similarity in the behavior of pollen and ovules.

In the F_1 plant $t=0.80$. The mean value of t in F_2 was 0.77. Nine F_3 progenies showed means ranging from 0.54 to 0.82.

The percentage of waxy pollen in F_2 and F_3 plants with two dominant *Wx* genes was much more variable than in plants with one *Wx*.

The greater variability in the percentage of waxy pollen grains in plants with two *Wx* genes as compared with those with one *Wx* is ascribed to individual variations in autosyndetic attraction.

In plants with one *Wx*, where there was no possibility of variation in the ratio of autosyndetic pairings, the observed intraprogeny variability of percentage of waxy pollen could all be attributed to sampling errors. In plants with two *Wx* genes, where variations in autosyndesis would change the percentage of waxy pollen, the variation could not be ascribed to chance.

Correlations between the percentage of waxy pollen and the percentage of waxy ovules gave supporting evidence of variation in the coefficient of autosyndesis. In the plants with one *Wx* gene there was no correlation, while in the plants with two *Wx* genes the correlation was significant.

There is fairly good evidence that the variations in autosyndesis are inherited.

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DICTYOSTELIUM DISCOIDEUM, A NEW SPECIES OF SLIME MOLD FROM DECAYING FOREST LEAVES¹

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INTRODUCTION

In the course of studies made in this laboratory on the amoeboid population of soil and decaying vegetable matter, myxamoebae in large numbers have been regularly encountered. Thom and Raper (8)³ reported that the amoeboid stage of some Myxomycetes formed a normal part of the microbiological population of field soil and decomposing crop residues, and could readily be isolated and grown in artificial culture. Following this, Raper and Thom (6) studied the distribution of the Acrasieae in soil and reported *Dictyostelium* as a common component of the soil population from widely scattered areas and from many types of soil, while *Polysphondylium* was repeatedly isolated from vegetable remains, particularly forest litter, and occasionally from soil.

These results were in close agreement with earlier work by Krzemieniewski (3) and extended her observations in this field. It was reported previously (6) that the vast majority of cultures of *Dictyostelium* isolated apparently belonged to the single species *D. mucoroides* Bref. Continued studies and isolations since that time have shown an equally large proportion belonging to that species and support the writer's earlier findings. However, not all cultures isolated could be identified with *D. mucoroides*. A form closely agreeing with the description of *D. sphaerocephalum* (Oud.) Sacc. and March., as given by Olive (4) in his comprehensive study of the group, has been occasionally obtained from decaying forest litter. *D. purpureum* Olive has been isolated once, from decaying sphagnum. Still another form has been isolated that does not agree with the description of any published species and differs fundamentally in some respects from the other members of the genus. It has seemed desirable, therefore, to describe it as a new species, to review briefly its life cycle, and to discuss at some length the formation and behavior of certain structures not seen in other species.

TECHNICAL DESCRIPTION

Dictyostelium discoideum, n. sp.

Soris griseo-albis vel citrinis, rotundatis, apiculatis, plerumque 125 μ -300 μ diam.; sorophoris griseo-albis, ex discis expansis oriundis, basi rigidis, ad apicem tenuibus, flexuosis attenuatis, 1.5-3 mm altis; discis basilaribus cellularibus, conicis, bases sorophororum circumvallentibus et sustentantibus, 150 μ -400 μ diam.; sporis anguste ellipticis, hyalinis, 6 μ -9 μ \times 2.5 μ -3.5 μ .

¹ Received for publication Nov. 19, 1934; issued April 1935.

² The writer is indebted to Charles Thom, Division of Soil Microbiology, for valuable counsel and criticism regarding the work, and to Edith K. Cash, Division of Mycology and Disease Survey, for preparing the Latin diagnosis.

³ Reference is made by number (italic) to Literature Cited, p. 147.

Hab. in foliis putrescentibus in silva decidua, North Carolina.

Colonies grown on hay and dung agar; sori grayish white to pale lemon yellow, rounded, apiculate, commonly 125μ 300μ in diameter, with larger and smaller specimens frequent; sorophores grayish white, arising from expanded disklike bases, upright, rigid below, 30μ – 80μ in diameter, tapering to thin flexuous above, 5μ – 15μ in diameter, 1.5–3 mm high, less commonly longer or shorter; basal disks cellular, conical, surrounding and supporting bases of sorophores, 150μ – 400μ in diameter; spores narrowly elliptical, hyaline, 6μ – 9μ by 2.5μ 3.5μ .

Isolated from decaying leaves from deciduous forest, North Carolina.

One of the most striking characters of this species, which is only suggested in the mature sorocarp ⁴ by a trail of slime leading away from the base, is the peculiar behavior of the pseudoplasmodium. In other described species of *Dictyostelium*, three of which, *D. mucoroides*, *D. sphaerocephalum*, and *D. purpureum*, the writer has studied in culture, the fruiting stalk is produced from the point where the myxamoebae congregate. In this species the myxamoebae come together to form an aggregate, or pseudoplasmodium, as in the other and more common forms, but in the ordinary laboratory culture, instead of developing into a sorocarp immediately, the myxamoebae compact themselves together to form an elongated cylindrical mass which moves as a unit across the agar plate for a greater or less distance before pausing to complete its cycle of development. The formation, structure, and behavior of this "migration pseudoplasmodium" will be considered in greater detail later.

ISOLATION AND CULTURE

Dictyostelium discoideum was isolated from decaying leaves collected in a hardwood forest of the North Carolina mountains in the summer of 1933. The dominant trees were beech, birch, oak, and buckeye, and the sample consisted largely of the partially decomposed leaves of these trees, together with some weed residues. The sample had a pH of 4.65.

In isolating the organism, culture methods similar to those reported by Raper and Thom (6) were used. The sample was ground in a clean mortar with approximately 5 parts of sterile water, and the resulting suspension was streaked upon mannite agar plates. The plates were incubated for 3 weeks at 18° to 20° C. At the end of this time spores from sori uncontaminated by fungi were transferred to

⁴ Following Zopf's use of the terms "sorus" for the spore mass and "sorophore" for the supporting structure, or stalk, Harper (2) introduced the term "sorocarp" to include the whole fruiting structure

EXPLANATORY LEGEND FOR PLATE I

A.—Mature sorocarp photographed from the side, showing the typical lemon shaped sorus, the erect, evenly tapered sorophore, and the expanded basal disk $\times 15$

B.—Spores. $\times 900$

C.—Vegetative myxamoebae growing in a bacterial colony, killed and stained with rose bengale and photographed in situ on the culture plate $\times 250$

D.—Vegetative myxamoebae stained as in C and photographed in higher magnification, showing bacterial cells in the surrounding medium. $\times 900$

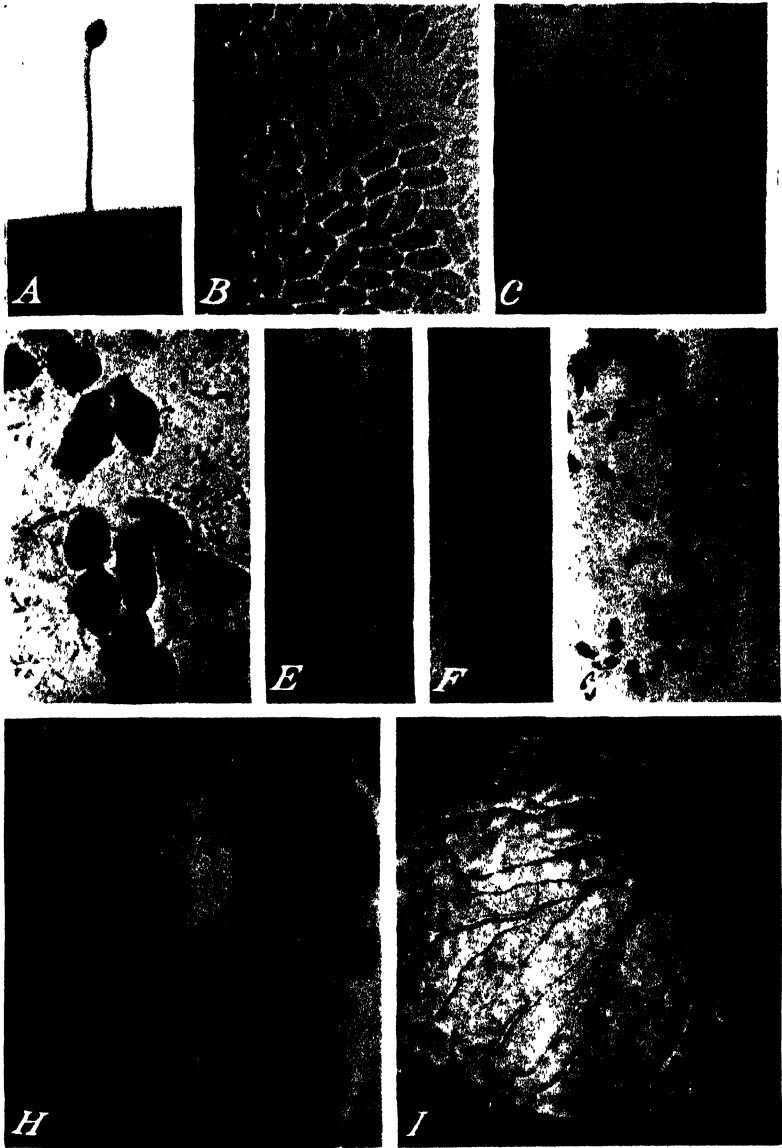
E.—Early stage in the orientation and aggregation of myxamoebae to form a pseudoplasmodial stream. In the lower part of the picture but little orientation of the myxamoebae is evident and aggregation is just beginning, while above a definite stream is already formed $\times 250$

F.—A slightly later aggregation stage, but one in which the myxamoebae remain in a single layer. Then elongate Limax form and rather uniform orientation are shown. Movement is toward the top of the photograph. $\times 250$.

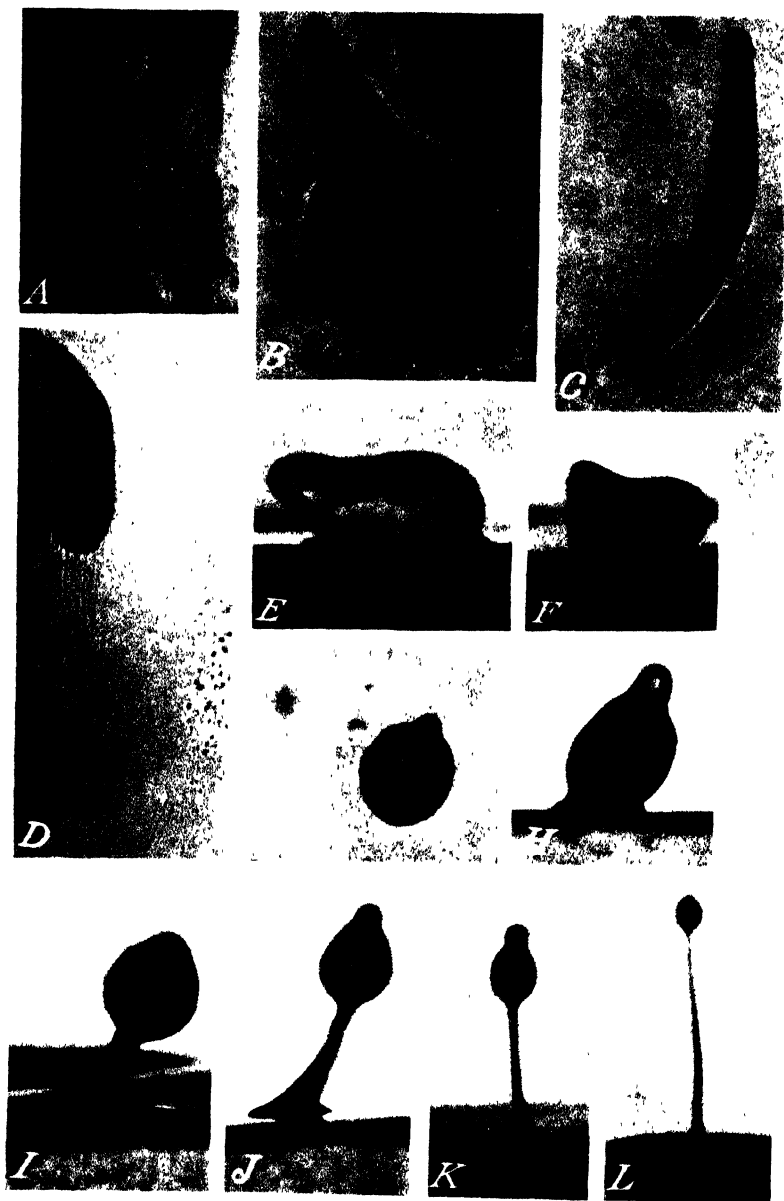
G.—A somewhat later stage in aggregation of the myxamoebae, which here have become compacted together to form a ropelike structure $\times 250$.

H.—Entire pseudoplasmodium with streams of myxamoebae radiating in all directions from the aggregation center shown as an irregular darkened area. Note that the streams can be traced directly to regions of heavy bacterial growth, which appear as darker areas in the photograph $\times 15$

I.—Somewhat later stage of aggregation, showing a large pseudoplasmodium surrounding a smaller one. In an earlier stage the entire colony was flowing toward a common center, upper right, then for some reason a main stream was severed, and as a result two fruiting masses will develop $\times 15$.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



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fresh plates of hay- and of dung-infusion agar adjusted to a pH of 6.0. The organism has been grown continuously upon these media and in Petri-dish cultures since that time. Transfers have been made at intervals of 2 to 3 weeks, although it is not necessary to transfer so frequently to maintain the stock culture. Cultures have been grown for the most part in a dark incubator at a temperature of 18° to 20°.

In photographing and in measuring the dimensions of myxamoebae and in studying their relation to one another in the pseudoplasmodium it is essential to disturb the organisms as little as possible. It is particularly difficult to obtain and to maintain a true picture of them as they naturally occur in culture. If they are transferred to slides they change shape and position during the process, and if they are observed on the agar plate the concentration of light and heat when high powers are used induces the same changes. To obviate these difficulties as far as possible, photographs and measurements of myxamoebae have been made of specimens killed and stained in situ with rose bengale⁵. This preparation kills the organisms immediately and stains them a darker red than the underlying medium, so that they stand out clearly and their actual form and position, as they occur in culture, are preserved. However, the stain has a decided disadvantage in that it stains the whole organism indiscriminately and does not permit cytological study. Only the figures shown in plate 1, *C* to *G*, are photographs of myxamoebae thus killed and stained in situ; other photographs are of unstained, living material. All the photographs were made with a Leitz Makam camera.

VEGETATIVE STAGE

When large numbers of spores (pl. 1, *B*) are planted upon fresh dung- or hay-infusion agar plates, germination begins within the first day and continues for 2 or 3 days thereafter. It is characterized by a swelling of the spore contents and a longitudinal splitting of the spore case, from which the protoplast emerges as a myxamoeba. The process is similar to that in *Dictyostelium mucoroides* and agrees closely with Olive's figures (4, pl. 6, figs. 42, 44).

In the vegetative stage following germination, the myxamoebae are quite hyaline and finely granular, and show little differentiation into endoplasmic and ectoplasmic regions. They are uninucleate and

⁵ One-percent rose bengale, or erythrosin, in 5-percent solution, to which is added a trace of calcium chloride

EXPLANATORY LEGEND FOR PLATE 2

- A* - A pseudoplasmodium in approximately the same stage of development as that shown in plate 1, *I*, with streams of myxamoebae leading in from the bacterial streaks on either side of the aggregation center $\times 15$
- B* - A migrating pseudoplasmodium leaving a bacterial colony $\times 50$
- C* - Migrating pseudoplasmodium. The transverse line in the anterior end marks the point of contact with the agar surface. $\times 50$
- D* - Migrating pseudoplasmodium with a slime streak marking its former path. Another slime streak cuts diagonally across the lower right-hand corner of the photograph $\times 50$
- E* - Side view of a pseudoplasmodium just before it ceases migration. $\times 50$
- F* - Side view of a pseudoplasmodium in which movement has ceased and sorocarp formation is beginning. $\times 50$
- G* - Similar stage of development as that shown in *F*, but viewed from above $\times 50$
- H* - Early stage in formation of the sorocarp. Mass of myxamoebae becoming divided into spore- and sorophore-forming regions $\times 50$
- I, J* - Successive stages, showing the sorogenic mass ascending the sorophore as it develops $\times 50$
- K* - Still later stage, showing a well-formed apical papilla in which active sorophore formation is taking place $\times 30$
- L* - Mature sorocarp. $\times 16$.

regularly possess a single contractile vacuole, which occupies a posterior position in the cell. Food vacuoles and vacuoles surrounding foreign bodies are often clearly evident.

In shape the myxamoebae vary widely. They are not infrequently elongate or of irregular shape, owing to the presence of extended pseudopods, but are more commonly rounded or broadly triangular. Plate 1, *C* and *D*, illustrates clearly this condition. The photographs are of myxamoebae killed and stained with rose bengale and photographed in situ on the agar plate, and thus faithfully show them as they appear when actively vegetating. In hanging-drop cultures, the myxamoebae are more irregular in outline and frequently show few to many thin, finely pointed pseudopods, particularly in the anterior region. In size the vegetative myxamoebae vary from 12μ to 20μ by 8μ to 12μ , with the majority about 14μ to 16μ by 9μ to 11μ . Measurements in such amoeboids are subject to rapid change with variations in shape, however, and cannot be relied upon with the same confidence as measurements of spores and other definite walled structures. It is obvious that an amoeba, while remaining constant in volume, would show, when flattened and actively moving, entirely different dimensions from those it would show when rounded and relatively quiescent.

The present studies add little to the controversial subject of the nutrition of myxamoebae. The vegetative myxamoebae of *Dictyostelium discoideum* reach their maximum development in bacterial colonies, outside the limits of which they occur as scattered individuals only. The writer has not yet been able to determine satisfactorily whether the greater amount of moisture in the bacterial colonies induces the concentration of the myxamoebae there, or whether the myxamoebae feed upon the products of bacterial metabolism or utilize substances in the substratum set free by bacterial activity, or whether they feed directly upon the bacterial cells. It is not uncommon to see myxamoebae with vacuoles enclosing bacteria, but to determine whether the bacteria are undergoing digestion or are merely present as so much inert material, as Olive (4) has indicated, is quite another matter. Pinoy (5), working with *D. mucoroides* and *D. purpureum*, and later Skupienski (7), working with the former species only, have reported the presence of bacteria as essential to the growth of these species. Pinoy considered the myxamoebae as parasitizing the bacterial colonies, while Skupienski believed that a symbiotic relation exists. Just what the relation is between the two in *D. discoideum* the writer is not prepared to say; certainly the myxamoebae of this species are not hindered in any way by the presence of a fair growth of "contaminating" bacteria.

Under favorable culture conditions the vegetative stage, that is, the period from spore germination to the beginning of pseudoplasmodium formation, lasts several days, and during that time the myxamoebae increase tremendously in number. The first pseudoplasmodia appear in 3 to 4 days, and others continue to develop for a similar period. The two stages, vegetative and fructifying, are thus seen to proceed simultaneously for a time in the same culture. Cell division of the vegetating myxamoebae of this species has not been studied.

AGGREGATION STAGE

Following a vegetative period, the myxamoebae congregate toward definite centers and form aggregations preparatory to fruiting. The process here agrees so closely with that in other *Dictyostelia* to which Harper (2) and Olive (4) have given such detailed study that it is only necessary to discuss it briefly. The first evidence of aggregation is the appearance of certain areas of closely packed myxamoebae. The stimuli involved in this aggregation have never been determined, but these strategically placed individuals apparently exert some stimulus which reaches myxamoebae in the surrounding area and causes them to become elongated and oriented in the direction of these centers and to flow toward them. Gradually the orientation proceeds outward and the colony of aggregating myxamoebae takes the form of definite streams converging toward definite centers. Plate 1, *E* to *G*, shows different stages in this process.

The formation of a pseudoplasmodial stream is shown first by the orientation and aggregation of scattered myxamoebae (pl. 1, *E*). In the outer edges of the area involved they appear only slightly different from vegetative forms and show little orientation, whereas toward the center of the developing aggregate the orientation is much more pronounced, the myxamoebae are crowded together, and the pseudoplasmodial stream is an accomplished fact. Plate 1, *F*, shows a slightly later stage, in which the myxamoebae are definitely out of the vegetative condition and are actively moving toward the aggregation centers. However, they are not compacted together to any particular degree and still remain in a single layer on the agar surface. The degree of elongation of the aggregating myxamoebae is shown here. In higher magnifications they are clearly differentiated into endoplasmic and ectoplasmic areas, the latter occupying a broad band at the anterior end of the myxamoebae. As the stream becomes slightly older, the myxamoebae are interlocked and overlap to such a degree as to form a compact ropelike mass (pl. 1, *G*).

The three photographs (pl. 1, *E-G*), although not of the same stream nor even of the same aggregate, are fairly typical of successive stages in the orientation of the myxamoebae and the formation of the pseudoplasmodium, and at the same time fairly represent sections of the stream successively nearer the aggregation center. All three photographs were made near the tips of streams, as it was quite impossible to photograph the larger streams near the centers and show details of individual myxamoebae.

Plate 1, *H* and *I*, and plate 2, *A*, show a lower magnification of entire aggregations. A pseudoplasmodium with streams of myxamoebae radiating in all directions from the center, which appears as a darkened irregular area, is shown in plate 1, *H*. The streams can be traced directly to regions of heaviest bacterial growth, which appear as darker areas in the photograph. Plate 2, *A*, which was taken at about the same stage in development, shows this condition equally well. The myxamoebae are being drawn primarily from the two bacterial streaks shown on either side of the aggregation center. The two pseudoplasmodia shown in plate 1, *I*, represent a later stage of development, in which many small streams have merged to form larger streams which appear as definitely raised and ropelike struc-

tures. The streaming and aggregation of the myxamoebae continue until a compact, elongate cylindrical mass is formed.

In plate 1, *I*, is further shown a very large pseudoplasmodium surrounding, as it were, a much smaller one, and in this case it is obvious that in an earlier stage the whole colony of myxamoebae was flowing toward a common aggregation center. Then, from some cause either foreign to or within the pseudoplasmodium or its component cells, a new center appeared, and as a result two fruiting masses will develop, each producing a sorocarp. This may well raise the question of just what determines the size of an aggregation. As Van Tieghem (9), Olive (4), Harper (2), and others have shown, the myxamoebae that make up a pseudoplasmodium retain their individuality, are mechanically separable, and react more or less independently of one another; none the less it is equally apparent that there is some stimulus or force which influences and determines what myxamoebae shall go into one aggregation and what into another, and consequently how large the fruiting structures shall be. In an even culture of myxamoebae, what determines whether there shall be more and smaller or fewer and larger sorocarps? For the present the whole subject must remain a matter of conjecture.

The developing pseudoplasmodium may be of almost any conceivable shape. In a fresh culture with an even distribution of myxamoebae, the form is typically radially symmetrical. But in an older culture where previous aggregations have already withdrawn the myxamoebae from certain areas, or in fresh cultures where there is an uneven growth of amoeboids, the centers once formed draw the myxamoebae from wherever they can. A resulting pseudoplasmodium may consist of a single extended stream, or of two streams extending out in opposite directions, or may be triangular as myxamoebae are drawn from a sector uninfluenced by previously developing masses, or even crescent-shaped as it forms at the periphery of a bacterial colony.

MIGRATION STAGE

Thus far the development of *Dictyostelium discoideum* follows closely that of other species of the genus. But at this point a stage occurs which does not appear in any of the Acrasieae described. The myxamoebae come together to form pseudoplasmodia which, instead of initiating a sorophore formation at once, gradually become transformed into compact, elongated cylindrical masses by the continued streaming and crowding together of their component myxamoebae. Sorophore formation is delayed, and between the appearance of the compact cylindrical pseudoplasmodium, as described above, and the beginning of sorophore formation, a period ensues during which the pseudoplasmodium moves as a unit over the surface of the agar until it reaches a suitable location for sorocarp formation. As shown earlier in this paper, the myxamoebae grow most luxuriantly in bacterial colonies, and the pseudoplasmodia are regularly formed within the limits of these colonies; thus the migratory stage may well be a demonstration of negative hydrotaxis, that is, a concerted movement on the part of the pseudoplasmodium to reach a drier situation in which to develop the fruiting structure. Plate 2, *B*, shows such a pseudoplasmodium leaving a bacterial colony.

In shape the typical migrating pseudoplasmodium is elongate cylindrical, with the anterior end tapering to a greater or less degree and the posterior end rather blunt and somewhat flattened horizontally (pl. 2, *C*). However, variations from this form are not uncommon; the moving mass may be longer and narrower, with a less pointed tip, or shorter and relatively thick, with the anterior end rounded rather than pointed. It may be either straight or slightly curved (pl. 2, *C*). As it moves, the greater portion of the mass is regularly in intimate contact with the agar surface, but with the pointed frontal portion slightly raised above it; the transverse line in the upper half of the pseudoplasmodium (pl. 2, *C*) marks the point where contact with the agar surface is interrupted. In long narrow specimens the mass may be raised in the central portion as well.

In size the migrating pseudoplasmodia vary widely, both as to relative dimensions of length and breadth, as pointed out above, and as to total volume. But in well-developed cultures on hay-infusion agar, which have been incubated for 5 or 6 days at 18° C., typical specimens show dimensions of about 0.8 to 1.2 mm by 0.15 to 0.25 mm, with extremes measuring as much as 2.0 mm in length and as little as 0.4 to 0.5 mm by 0.07 to 0.1 mm. With some exceptions, volume is clearly dependent upon the extent of the aggregating mass from which the migratory structure developed, but the factors which determine the relative proportion of length to breadth are not so obvious.

If a migrating pseudoplasmodium is crushed in a drop of water under a cover slip, the entire mass is seen to be composed of apparently similar and undifferentiated cells. There is not the slightest indication of a stalklike structure, and the myxamoebae readily and quickly separate from one another. They are at first rounded, almost spherical, with diameters averaging 8.5μ to 9μ , but after the colony of dissociated myxamoebae has been allowed to stand for 15 to 30 minutes the organisms tend to become more elongated, show a differentiation into the endoplasmic and ectoplasmic areas, and begin a slow but perceptible amoeboid movement which marks the beginning of a new organization, or reorganization, of the myxamoebae into new fruiting masses. On the other hand, if a similar pseudoplasmodium is killed and stained in situ and then removed from the agar surface and broken apart, the myxamoebae are seen to be not round but elongated and somewhat angular in outline. As would be expected, the long axes are oriented in the direction of movement. The average dimensions of the myxamoebae at this stage are about 12μ to 13μ by 5μ to 6μ . Movement of the migrating pseudoplasmodium as a unit is obviously accomplished by the concerted and, at the same time, independent action of the countless individual myxamoebae of which it is composed.

In the Acrasieae the production of slime is characteristic, but in *Dictyostelium discoideum* it manifests itself in an earlier stage and in a somewhat different manner. As the migrating pseudoplasmodium moves over the agar surface it produces and leaves behind a trail of slime which persists and can be seen leading away from any migratory mass or mature sorocarp. The material is quite tenacious, for when a sorocarp or pseudoplasmodium is removed, invariably a portion of the slime streak, often 2 to 3 cm in length, remains attached to it.

Plate 2, *D*, shows a pseudoplasmodium with a trail of slime clearly marking the path it has traveled; cutting diagonally across the lower right-hand corner of the same photograph is the trail of another pseudoplasmodium. Embedded in the slime streaks are scattered myxamoebae that have been left behind; microscopically they appear as vacuolated cells of irregular shape. As will be seen later, the number of myxamoebae thus left behind varies with the rate of progress made by the migrating structure.

Regularly the migration pseudoplasmodium produced by an aggregating colony of myxamoebae remains as a unit as it creeps over the plate, and forms a single sorocarp. However, the mass not uncommonly divides, either leaving behind a mass of myxamoebae which produces an independent fructification or splitting into two masses each of which continues to move as a typical migratory structure. Secondary divisions of these pseudoplasmodial masses have occasionally been observed. In order to study their habits to best advantage, the migrating structures were removed from the cultures in which they grew and transplanted to fresh plates where they were less crowded and where their movements could be followed more easily.

In contrast to this splitting of a pseudoplasmodium into more or less equal masses, in older cultures there frequently occurs a gradual fragmentation of the entire structure. In such cases comparatively small masses of myxamoebae are cast aside, and these proceed to develop into small atypical sorocarps at the point where they are cut loose from the larger, parent structure. A large pseudoplasmodium may in this way give rise to a whole series of miniature fruiting bodies lining its path; and intermixed with the sorocarps countless myxamoebae remain stranded, either singly or in groups of varying size. Just what brings this condition about is not entirely clear, but it is no doubt influenced by the drying out of the agar and perhaps through the accumulation of waste products as well.

In cultures grown at 18° to 25° C. the migration pseudoplasmodia are formed and behave in the manner described above. When cultures are grown at 30° to 32° the pseudoplasmodia are formed in the same way but behave very differently. In such cultures the majority never leave the bacterial colony in which they are formed, but proceed to form sorocarps at the points where the aggregates develop. Sorocarps formed under such conditions, however, are not unlike those which develop from migrating pseudoplasmodia. Further studies are in progress concerning the growth and behavior of the species in response to changes in its environment.

SOROCARP FORMATION

Following the period of migration described above, the pseudoplasmodium ceases forward movement and builds an erect fruiting structure which, in accordance with Harper's usage, may be called the sorocarp. This development is shown in a series of photographs (pl. 2, *E-L*), all of which, with the exception of plate 2, *G*, are side views of successive stages.

The first evidence of this development is a relative shortening and thickening of the pseudoplasmodium. Forward movement of the mass becomes progressively slower until it ceases altogether. The myxamoebae, however, continue to crowd together, and the whole

structure takes on a more rounded appearance (pl. 2, *E*). In plate 2, *F*, this tendency has progressed further, and what has been the pointed anterior end now appears as a raised knob at one side; the whole colony is here becoming definitely transformed into an upright structure. Plate 2, *G*, shows a slightly more advanced stage as viewed from above. The structure presents a rounded, almost symmetrical form, with the apex of the developing sorocarp lifted above the mass.

In plate 2, *H*, the structure is more erect and is becoming definitely differentiated into sorophore and spore-forming parts, and it is in structures presenting this appearance that the early stages of sorophore formation are to be found. As development continues and the sorophore becomes longer and sufficiently strong to support the weight, the mass of myxamoebae slowly begins its ascent. In plate 2, *I*, this is clearly shown; the basal portion of the sorophore is complete and is securely anchored to the substratum, and the spore-forming mass is quite removed from it. This particular figure, however, is somewhat atypical in that it shows no pronounced apical region where sorophore formation is taking place. In the developing sorocarp of *Dictyostelium mucoroides*, as pointed out by Harper (2) and as seen in culture, the pseudoplasmodium extends down the sorophore as a band either applied to one side or coiled spirally around it, and may form a continuous stream between the substratum and the sorogenic mass. Such a condition has not been observed in *D. discoideum*; the pseudoplasmodium, or spore-forming mass, climbs the developing sorophore as a compact unit.

Plate 2, *J*, is a somewhat later stage and more nearly presents the usual picture. The apical region is composed of undifferentiated myxamoebae, which later become extremely vacuolated and contribute to sorophore development. The myxamoebae making up the bulbous mass below are destined to form spores; in fact, those nearest the periphery have already become differentiated at this stage. The fact that the stalk and base in this figure are heavier in proportion to the size of the sorocarp as a whole than usually is the case is due to exposure to the dry air of the laboratory preparatory to and during the process of photographing. A still more advanced stage is shown in plate 2, *K*, in which the relative proportions of parts in the developing structure are more nearly normal. The well-formed apical papilla indicates that active sorophore formation is in progress. Mature sorocarps are shown in plates 1, *A*, and 2, *L*, all the myxamoebae having become differentiated either into stalk cells or spores.

The mature sorocarp is typically a symmetrical structure, consisting of a disklike cellular base which envelops and supports the base of the stalk, an unbranched sorophore of similar nature which is relatively thick and rigid below but tapers rather evenly to a thin terminal region above, and a rounded apiculate or lemon-shaped sorus. Such an expanded disklike base has not hitherto been described in the Acrasieae. When seen from above, this basal disk is roughly circular in outline, and the upright sorophore arises perpendicularly from the central region of it (pl. 3, *D* and *F*). When viewed from the side, the structure presents a somewhat conical appearance, with a more or less centrally placed cuplike depression into which the base of the sorophore fits (pl. 3, *E*).

What actually happens is that the basal portion of the upright sorophore is first formed and then the basal disk is built outward from it

at the same time that it continues to be built upward. When a young fruiting mass such as that shown in plate 2, *H*, is crushed, it is found that the basal part of the sorophore is in process of formation, and extending outward from it on all sides a band of myxamoebae are cut off by what is apparently a slime sheath not unlike that which extends upward through the mass during sorophore formation. Inside this basal sheath, the cells nearest the upright sorophore are differentiated first and later those further removed are differentiated.

At the periphery of the completed disk the highly vacuolated and horizontally flattened cells form a single layer (pl. 3, *F*). The disk becomes thicker in cross section nearer the sorophore, with the cells piled one above the other. Bordering the sorophore a sleeve-like layer of disk cells extends upward for some distance (pl. 3, *E*). The whole structure is enclosed in an envelop of slime which is continuous with that of the sorophore, and extending out from one side of the disk is the trail of slime laid down by the migrating pseudoplasmodium, shown in plate 3, *D*, as a thin line curved about the base.

The sorophore is composed of a slightly bulbous base (pl. 3, *D*), which is enclosed in the basal disk described above, and a cylindrical, elongated tapering shaft which supports the sorus. Its structure is like that of *Dictyostelium mucoroides* (pl. 3, *B*, *C*, *D*, and *F*), although it is more rigid and is almost invariably formed perpendicular to the substratum to which it is attached. Its greater rigidity is due to two factors: (1) In relation to height it has a greater basal diameter and the degree of tapering is greater than in *D. mucoroides*, thus adding to its strength; and (2) the expanded cellular base rests squarely upon the substratum, thus giving the sorophore a much firmer support than in the more common species, where it is anchored by slime only.

As noted above, the bulbous base of the sorophore is formed first, and extending upward from it in the central region of the young sorocarp is a tubular slime sheath which is formed in advance of the vacuolated cells and in which these cells become packed as growth of the sorophore progresses. This sheath is continuous with the sorophore below and the greater part of it is of the same diameter; the terminal region, however, is regularly expanded into a funnellike structure. A similar structure is present in other *Dictyostelia*, and figures of it in *Dictyostelium mucoroides* have been given by Brefeld (1, pl. III, fig. 21) and Harper (2, pl. 6, fig. 14). Brefeld's figure shows the slime sheath formed for some distance beyond the enclosed, vacuolated cells; and, except in the absence of an expanded terminus, it agrees with structures commonly seen in young, rapidly developing sorocarps of *D. discoideum*. As to the origin of this sheath, it is not clear whether it is secreted by cells that are destined to become stalk

EXPLANATORY LEGEND FOR PLATE 3

A.—Terminal region of developing sorocarp, showing the funnel-shaped sheath formed in advance of sorophore formation. The apical papilla and the region surrounding the top of the sorophore are composed of amoeboid cells. Below, spores are already differentiated. $\times 320$.

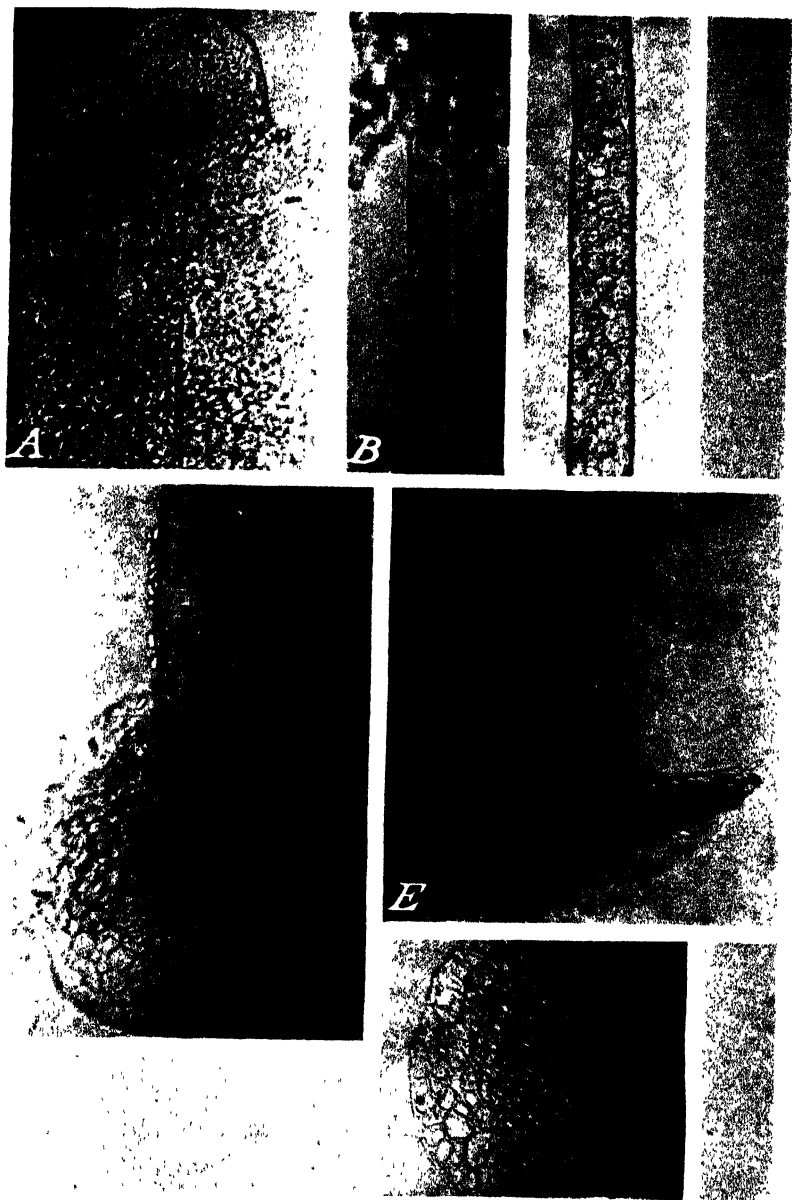
B.—Terminal region of a sorophore, consisting of cells in a single row or superimposed on other cells $\times 900$.

C.—Median region of a sorophore $\times 320$.

D.—Basal part of a sorocarp, showing the swollen base of the upright sorophore and the expanded basal disk from which it arises. The slime streak remained attached and is shown as a line curved about the base of the sorophore. $\times 320$.

E.—Basal part of a sorocarp, showing the conical shape of the basal disk and how the end of the sorophore fits into and is attached to it. $\times 320$.

F.—Basal disk as viewed from above, showing the end of the sorophore as a circular structure in the upper right corner of the figure $\times 320$.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

cells or by the surrounding cells, which later form spores. In either case it serves to separate the two groups of cells before there is any evident differentiation of either.

In plate 3, *A*, the terminal region of a more advanced sorocarp than those just under consideration is shown, and in it, in contrast to the condition in younger, rapidly developing structures, the expanded portion is just above the rounded, vacuolated cells, which later become compacted together to form a rigid sorophore. Above the developing sorophore is the apical papilla of undifferentiated amoeboid cells which contribute to its further extension. Just below and surrounding the terminal region of the sorophore are other undifferentiated myxamoebae; whether they all ultimately become spores or whether some of them join the apical mass and become stalk cells has not been determined.

In the lower part of the picture the outlines of spores already formed can be seen. Once the mass of myxamoebae has become separated from the substratum (*pl. 2, I, J*) and is ascending the sorophore as it develops, the myxamoebae at the periphery of the mass in a centrally located band begin to be differentiated into spores, and as this sorogenic mass rises continually higher on the developing sorophore more and more spores are formed. If a sorocarp such as that shown in *pl. 2, K*, is studied in a drop of water, a band of spores is seen completely encircling the developing head; if as many spores as possible are washed away and the structure is crushed under a cover slip, it becomes evident that beneath the layer of spores is a mass of cells in stages of differentiation into spores and that around the sorophore is a cylinder of cells still amoeboid which carries the mass upward. Gradually the cells in the apical region are used up in sorophore formation, and those surrounding it become spores to complete the fruiting structure.

TABLE 1. *Comparative dimensions of parts of sorocarps of Dictyostelium discoideum from 3-week-old dung-agar cultures*

Size of sorophore						
Diameter of expanded base	Length	Portion above basal swelling		Terminal region		Diameter of sorus
		Diameter	Cells in cross-diameter	Diameter	Cells in cross-diameter	
μ	μ	μ	Number	μ	Number	μ
200	1,800	42	5-6	8.5	1, 1, and 2 ¹	225
200	2,350	38	5	9	do ¹	200
210	2,210	39	5	7.5	1	230
240	2,510	45	6	9.2	1 and 2.	275
205	2,250	41	5	7.5	1	240
185	2,050	32	4	7	1	225
210	2,420	37	5	8.5	1, 1, and 2 ¹	300
325	2,850	70	9-10	14	2	350
270	2,500	48	7-8	10.2	2	250
225	2,440	42	6	8.5	1 and 2	275
200	2,240	38	5.6	9	do	225
300	2,625	60	8	10	2	325

¹ The sorophore was usually 1 cell but occasionally 2 cells wide.

When sorocarps in large numbers are observed, one is impressed with the relation in size that exists between their different parts, for example, the diameter of the basal disk, sorophore, and sorus. This relationship is not exact, to be sure, but within limits it does exist. In table 1 are given the measurements of a group of sorocarps picked from dung-agar cultures 3 weeks old. Dimensions of the sori were taken in situ, and the sorocarps were then removed to slides, where other measurements were made. Roughly the ratio of the diameter of the expanded disklike base to the diameter of the sorophore just above the swollen base is 5 to 1 and the ratio of the diameter of the sorophore at this point to its diameter in the terminal region is likewise approximately 5 to 1. The relationship between length and diameter is not so regular, but in general the longer sorophores have the greater diameters, arise from the larger bases, and bear the larger sori.

In *Dictyostelium discoideum*, as in other Acrasieae, there is a striking relation between the diameter of the sorophore in the terminal region and the size of the sorus. And in fructifications where the sorophore terminates in a single row of cells, the shape of these cells varies with the volume of the spore mass. Harper (2) has made a detailed study of this matter in *D. mucoroides*, and the writer's observations on *D. discoideum* agree closely with his. If the sorus is very small the cells are narrow and elongated, 15μ to 18μ by 3μ to 3.5μ , or with the long and short diameters in the ratio of 5 to 1; if larger, the cells are more nearly isodiametric; and if still larger, the cells are horizontally flattened, 3μ to 4μ by 9μ to 10μ , or with the long and short diameters in the ratio of 1 to 3. In cases where the sori are especially large, the sorophores are 2 or rarely 3 cells wide and the cells are of approximately equal width and length. Thus it would seem that the size of the sorophore is the essential thing; it must be of a size and strength sufficient to support the spore mass, and the cells that go into the making of it become modified in such a way as to attain this objective.

Sorocarps regularly develop at right angles to the substratum or other support to which they are anchored. In the usual culture they are produced upon the agar surface and are built up vertically. If grown in inverted plates they are built vertically downward. If cultures are grown in uneven light and as a result migrating pseudoplasmodia climb up the side of the culture dish, the resulting sorocarps develop outward horizontally. In cultures grown in uneven light or temperature there is no pronounced inclination of the sorophores toward either light or warmer temperature.

True branching has not been observed in *Dictyostelium discoideum*. On several occasions structures have been seen which, under low magnification, appeared to be branched sorophores. But in each case, on closer examination, the "branched" structure was seen to be composed of two sorocarps complete in every detail, one of which was anchored to the substratum while the other was anchored to the upright sorophore of the first. The "branch" possessed a typical expanded base, which was somewhat curved around the supporting sorophore and was securely anchored to it by an envelop of slime. In regard to such compound structures the question arises as to whether the supporting and the supported sorocarps arise from the same colony of aggregating myxamoebae. The evidence indicates that this is not the case but that the branch originated in a later pseudoplasmodium which climbed up an established sorophore, just

as it would have climbed up the side of the culture dish, and then proceeded to form a sorocarp.

SUMMARY

A new species of *Dictyostelium* is described, for which the name *Dictyostelium discoideum* is proposed.

Following the aggregation of the myxamoebae, the pseudoplasmodium becomes a compact cylindrical mass and moves for a greater or less distance over the culture plate before developing a sorocarp. This migratory stage, which has not been previously reported, is termed the migration pseudoplasmodium.

The mature sorocarp differs from that of the more common species in possessing a cellular basal disk, which surrounds and supports the base of the sorophore. In addition, the sorophore is more rigid and tapers more evenly than in other species.

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EFFECT OF TEMPERATURE OF ARTIFICIAL DRYING ON DIGESTIBILITY AND AVAILABILITY OF NUTRIENTS IN PASTURE HERBAGE¹

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INTRODUCTION

Mechanical driers are being used in the artificial preservation of forage crops in certain sections of the United States. The future of this method of preserving crops for winter feeding depends largely on the development of machines that will dry forage efficiently and economically without affecting its nutritive value.

REVIEW OF PREVIOUS INVESTIGATIONS

As a result of research with a rotary triple-drum drier (Ardrier) Clyde (4, 5)² has been able to increase the efficiency and drying capacity of the drier by reducing the air flow and increasing the initial temperature of the heating gas. By increasing the initial temperature from an average of 932° to 1,451° F., it was possible to increase the rapidity with which the moisture was evaporated from the hay and to reduce the exhaust temperature from 291.7° to 237°. Barr (2, 3) working with the Louisiana station rotary single-drum drier reported that the lowest efficiencies (when drying green forage) were obtained when the initial gas temperature ranged from 1,100° to 1,200° F. and that the highest efficiencies were obtained at 1,850°. He found that with the highest initial heat the exhaust temperature maintained itself at 240° and the forage had a temperature of 198°. The extent to which the temperature of drying may be raised to effect a greater and more rapid evaporation of moisture without rendering valuable feed nutrients unavailable is not apparent. The experiment described herein was undertaken to determine the effect of increasing the temperature of drying on the nutritive value of the herbage.

Hart, Kline, and Humphrey (11) found that the nutrients of green alfalfa exposed to temperatures of 480° to 535° C. in a Koon-type drier for 40 seconds were no less digestible than those of similar alfalfa dried in the field. Woodman and his associates (20) concluded that the high nutritive value of young pasturage is not appreciably depressed by artificial drying with steam at 100° C. Pasture grass dried by direct heat in a kiln chamber at 115° for 3 hours was only slightly less digestible than similar herbage dried at the temperature of steam. They report that the grass, while in the chamber, at no time reached a temperature of over 90°.

¹ Received for publication Nov. 19, 1934; issued April 1935.

² Reference is made by number (italic) to Literature Cited, p. 163.

Watson and Ferguson (18) compared the digestibility of pasturage that had been dried at different temperatures in a band drier and in a pneumatic-conveyor drier with fresh green herbage. In the band drier, at an inlet temperature of 200° C., the herbage was dried to 10-percent moisture in 20 minutes with no effect on the digestibility of the various constituents, except on that of the crude protein, which was slightly depressed. With similar herbage, dried in the conveyor drier at a temperature of 600° for 15 seconds, a depression of the digestibility of the constituents of the grass was noted, particularly the digestibility of crude protein which was decreased markedly. Watson and Ferguson (19) reported that hay of excellent quality and high digestibility was made by artificial drying in a stack through which hot air was blown. The temperature of the hay was maintained at an average of 100° to 110° F. for 45 hours during the drying process. Honcamp (15) reported that artificial drying of grass at low temperatures caused no loss in the raw or digestible nutrients. When artificial drying was accomplished by hot fire gases a considerable reduction of protein digestibility occurred. He did not, however, specify what temperatures were used.

Several investigators (14, 16, 18, 20) have reported the digestibility coefficients of the nutrients in artificially dried young grass. In most instances the materials studied have been dried under different conditions. To facilitate comparison these coefficients are assembled in table 1.

TABLE 1.—Coefficients of digestibility of artificially dehydrated pasture herbage, as reported by several investigators

Treatment of grass	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash	Investigators
	Percent	Percent	Percent	Percent	Percent	Percent	
2-week-old grass dried in oven at 150° F. for 10 hours.	67.57	74.92	74.55	21.90	72.68	32.48	Hodgson and Knott (14).
7- to 10-day-old grass artificially dehydrated in Ardrier.	72.4	71.0	81.6	46.4	73.0	38.5	Newlander and Jones (16)
Young grass dried at 600° C. for 40 seconds.	65.90	58.94	75.96	55.76	68.00	-----	Watson and Ferguson (18)
Young grass dried at 100° C for 3 hours.	-----	78.2	80.4	73.7	81.0	-----	Woodman, Bee, and Griffith (20)

EXPERIMENTAL PROCEDURE

The pasture herbage used in this investigation was a mixture of English ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*), and white clover (*Trifolium repens*). The pasture was seeded in the early spring of 1933 on fertile bottom-land soil on the Western Washington Experiment Station at Puyallup. On April 17, after the seeding had made a good start, 300 pounds of sodium nitrate and 450 pounds of superphosphate per acre were applied. Due to the abundant and well-distributed rainfall a luxuriant and uniform growth was maintained throughout May, June, and July, when the samples were taken. The herbage was rather free from weeds and contained approximately 20 percent of white clover and 40 percent of each of the ryegrasses.

The pasture was divided into three plots, one of which was cut each week. The grass represented 3 weeks' growth when cut. Grass that was to be fed green was cut each day, run through a chopper, and fed twice daily. Grass that was to be dried, either artificially or in the sun, was cut as soon as the dew was off in the morning, collected immediately, run through a silage cutter, and spread out to wilt overnight, and dried the following day. Curing in the sun was accomplished by spreading the grass thinly on a canvas and exposing it to the sun and indirect sunlight for 18 hours on the average. The grass was turned occasionally to insure uniform exposure, but was not exposed to rain or dew and suffered little or no loss of leaves. Artificial drying was accomplished with an experimental direct-heat rotary single-drum drier.³ Diesel oil was used as a source of fuel. The temperature of the gas at the outlet end of the drier was used as an index of the drying temperature, grass being dried at four different temperatures. The inlet temperature and the rate of speed at which the grass passed through the machine remained about the same for the four different rations dried, the only varying factor being the amount of material in the machine. The amount of material in the machine at any one time regulated the exhaust-gas temperature. The grass was dried at outlet temperatures of 250°, 300°, 350°, and 400° F., with an average variation of not over 5° above or below these temperatures. The wilted herbage, having an average dry-matter content of 19.5 percent, passed through the drying machine in from 2 to 5 minutes.

Six digestion and mineral-balance trials were conducted with a group of three sheep, each of the six rations of pasture grass described above constituting a trial. The sheep were range yearling wethers of unknown history. They were conditioned to the metabolism crates and rations before the experimental trials were begun. During each trial the three sheep were confined to individual metabolism crates (fig. 1). The crates were patterned after those described by Forbes (6) but with certain modifications to meet specific needs. Each crate had a floor area of 20 square feet. A manger and yoke were built in one end of the crate so that the sheep could be stanchioned while eating (figs. 2 and 3). Water and salt were accessible through openings in the rear end of the crate (fig. 2). The crate had two false bottoms, one of ¼-inch mesh galvanized wire on which the sheep stood and which permitted the excreta to pass through, and the other of ¼-inch mesh galvanized wire which caught the feces and allowed the urine to pass through. The urine was caught on a funnel-shaped pan and conveyed to glass receptacles (fig. 4).

The procedure outlined by Forbes and Grindley (7) for conducting digestion trials was followed in this experiment. The digestion trial for each type of feed lasted 3 weeks. The preliminary period was 7 days, while the collection period continued for 14 days. The sheep were weighed for 3 successive days at the beginning and end of each trial.

The freshly cut herbage was thoroughly mixed, and weighed amounts were fed to the sheep. At the same time duplicate samples were taken for dry matter and chemical determinations. In the trial

³ The writers wish to express appreciation for cooperation given by M. S. Grunder, agronomist, Western Washington Experiment Station, who built the artificial drying machine and supervised its operation in the preparation of the dried grass used in the experiment.

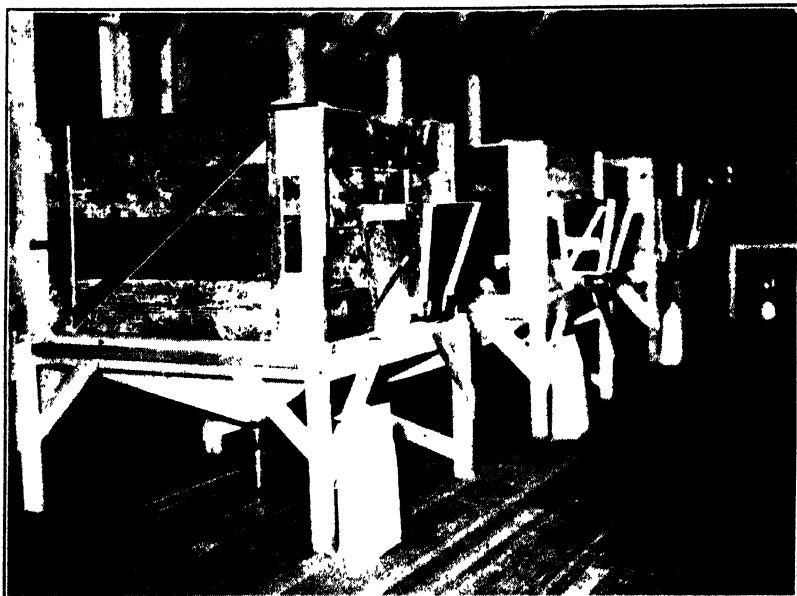


FIGURE 1 Individual metabolism crates used for sheep

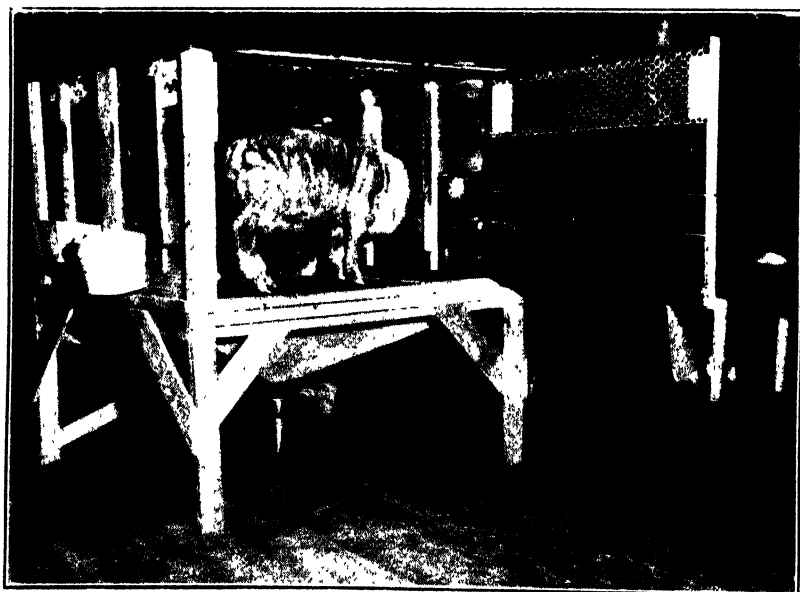


FIGURE 2.—Metabolism crate open and showing salt and water receptacles, and wether stanchioned as for feeding.

with the freshly cut herbage the refused feed was weighed daily and sampled for dry matter and chemical analysis. There was no refused feed during the trials in which the sun-cured grass or the artificially dried grass rations were fed.

At the beginning of the trials in which the sun-cured grass ration and the four artificially dried grass rations were fed, a sufficient amount of the material from each ration to last the entire trial was weighed out, thoroughly mixed, and divided into three piles. Weighed individual feedings, which were the same throughout the trial, were made up from the three piles and stored in paper bags. At regular intervals samples from each of the three piles were saved for chemical analysis and for color determinations.

The sheep were fed twice daily, at which time they were fastened in the yokes to avoid losing feed in the crates. Fresh spring water containing negligible amounts of calcium and phosphorus was provided at all times and salt was fed daily.

At the end of the experimental day the feces were collected, thoroughly mixed, weighed, and 50 percent of the daily excretion was saved for chemical analysis.

The samples were collected in a thymol-coated receptacle and stored in a refrigerator at 22° F. The daily excretion of urine was mixed, weighed, and a 20-percent aliquot sample saved for analysis. The samples were stored in thymol-coated air-tight glass bottles and preserved by adding a thymol chloroform mixture at the rate of 2 cc per liter. A 10-percent acetic acid solution was added at the rate of 1 cc per liter to prevent precipitation. At the close of the collection period the daily aliquots of urine and feces of each sheep were mixed and sampled for chemical analysis. The analyses of the feeds, feces, and urine were made by the usual methods as outlined by the Association of Official Agricultural Chemists (1). The color determinations

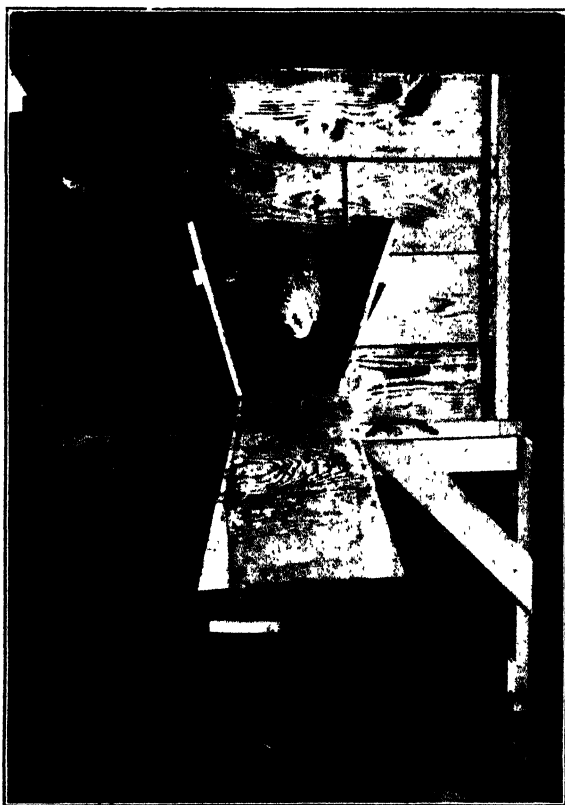


FIGURE 3 Metabolism crate with wether stanchioned and the front of the manger down to facilitate cleaning.

of the samples of the dried herbage were made according to the method described by Nickerson (17).⁴

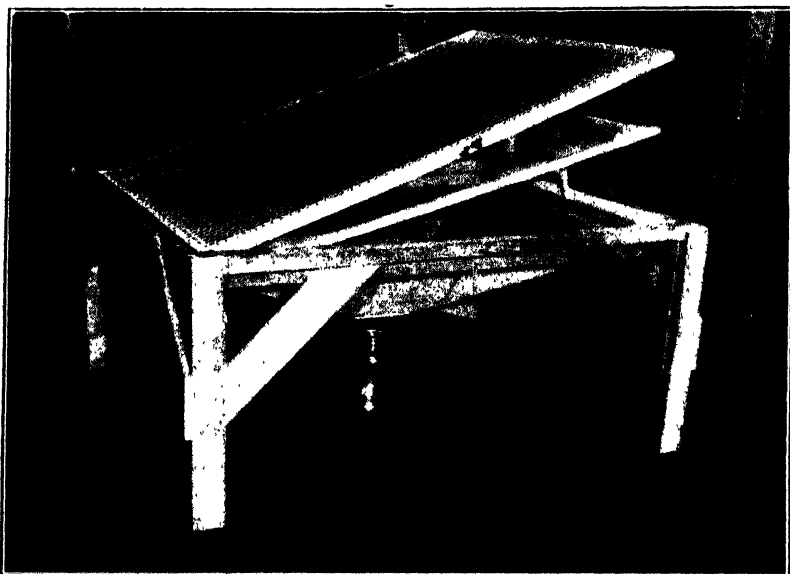


FIGURE 4.—Platform of metabolism crate showing the upper screen on which the animal stands, the lower screen which retains the feces and allows the urine to pass through, and the sloping metal bottom which drains the urine into the glass receptacle.

EXPERIMENTAL RESULTS

COMPOSITION OF FEED

The chemical composition of the six rations of pasture grass used in this experiment is shown in table 2.

TABLE 2.—Chemical composition of rations of pasture grass used in the experiment (dry-matter basis)

Rations	Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash	Nitrogen	Calcium	Phosphorus	Ca/P
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Ratio
Green grass, no treatment	23.90	10.36	21.46	3.92	45.99	12.27	2.62	0.761	0.484	1.57
Sun-cured grass, exposed 18 hours	92.16	18.82	20.54	3.46	44.62	12.56	3.01	.732	.647	1.13
Grass artificially dried at exhaust temperature of—										
250° F.....	92.41	17.50	20.21	4.59	46.52	11.18	2.80	.729	.516	1.41
300° F.....	92.72	17.78	20.98	5.09	43.79	12.36	2.84	.702	.555	1.37
350° F.....	92.56	18.19	20.98	4.23	44.90	11.67	2.91	.670	.517	1.30
400° F.....	93.18	17.98	22.88	5.04	42.46	11.64	2.88	.699	.583	1.18

The various dried-grass rations are very uniform in composition, indicating that each sample was truly representative of the herbage. The slight increase in the crude-fiber content of the grass dried at the

⁴ Color determinations were made by the Bureau of Agricultural Economics, U. S. Department of Agriculture.

exhaust-gas temperature of 400° F. may indicate slight burning of the more leafy material as it passed through the machine. Upon looking into the exhaust end of the drier it was apparent that some burning was taking place at this high drying-temperature. The calcium and phosphorus content of this herbage is high but is in close agreement with that of other grass produced on adjacent fields at this station. (10, 13, 14.)

COMPOSITION OF EXCRETA

In table 3 is given the composition of the urine and feces of the animals used in the six metabolism trials. There is a high degree of uniformity in the composition of the organic and inorganic constituents of the excreta of the individuals during each of the test periods. The dry-matter content of the feces of sheep no. 1 was considerably lower than that of the others throughout each experiment. This condition did not, however, affect the composition of the dry matter of the feces or the ability of the animal to digest nutrients. The grass dried at 350° and 400° F. had a more laxative effect upon the animals than the other rations.

TABLE 3.—*Chemical composition of feces and urine of sheep receiving rations of green, sun-cured, and artificially dehydrated pasture grass dried at different temperatures*

GREEN GRASS, NO TREATMENT

Sheep no., and material analyzed	Dry matter	Crude protein	Crude fiber	Ether extract	Nitro- gen-free extract	Ash	Nitro- gen	Calcium	Phos- phorus
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Sheep 1.									
Feces.....	42.09	15.21	17.45	3.82	37.12	26.40	2.43	2.395	1.4111
Urine.....							1.01	.0017	.00122
Sheep 2.									
Feces.....	53.57	15.12	18.98	4.44	36.48	21.98	2.43	2.388	1.371
Urine.....							.839	.00418	.000936
Sheep 3.									
Feces.....	54.76	15.30	18.23	3.47	37.44	25.56	2.45	2.410	1.434
Urine.....							.914	.0091	.00079

SUN-CURED GRASS, EXPOSED 18 HOURS

Sheep 1.									
Feces.....	46.86	17.02	13.27	5.21	32.44	32.06	2.72	2.675	2.010
Urine.....							1.486	.00404	.000855
Sheep 2.									
Feces.....	53.80	16.53	14.16	4.45	32.59	32.27	2.64	2.698	1.971
Urine.....							1.256	.00113	.000927

GRASS ARTIFICIALLY DRIED AT 250° F.

Sheep 1.									
Feces.....	34.74	18.36	15.03	6.56	32.66	27.39	2.94	2.650	1.856
Urine.....							1.759	.00374	.00107
Sheep 2.									
Feces.....	55.37	17.30	15.86	4.02	34.63	28.19	2.77	2.788	1.913
Urine.....							1.332	.00176	.000911
Sheep 4.									
Feces.....	52.06	18.12	14.09	5.88	32.78	28.53	2.90	2.699	1.925
Urine.....							1.562	.00310	.00110

TABLE 3.—*Chemical composition of feces and urine of sheep receiving rations of green, sun-cured, and artificially dehydrated pasture grass dried at different temperatures—Continued*

GRASS ARTIFICIALLY DRIED AT 300° F

Sheep no., and material analyzed	Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash	Nitrogen	Calcium	Phosphorus
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Sheep 1.									
Feces	34 00	18 65	15 44	5 65	33 25	27 01	2 98	2 704	1 883
Urine							1 813	00247	00103
Sheep 2									
Feces	46 94	18 12	15 81	5 05	33 34	27 68	2 90	2 750	1 835
Urine							1 477	00199	000935
Sheep 4									
Feces	50 39	18 50	16 79	3 80	32 16	28 75	2 96	2 918	1 918
Urine							1 846	00215	00116

GRASS ARTIFICIALLY DRIED AT 350° F

Sheep 1									
Feces	27 36	20 66	14 91	6 07	32 01	26 35	3 31	2 537	1 749
Urine							1 837	00224	000935
Sheep 2									
Feces	41 64	19 87	14 96	4 03	34 46	26 68	3 18	2 575	1 768
Urine							1 531	0 344	000756
Sheep 4									
Feces	36 20	20 38	14 75	3 70	34 06	27 11	3 26	2 618	1 805
Urine							1 779	00296	000940

GRASS ARTIFICIALLY DRIED AT 400° F

Sheep 1									
Feces	30 05	24 93	14 13	4 00	32 62	24 32	3 99	2 193	1 572
Urine							1 499	00176	00127
Sheep 2									
Feces	39 77	21 06	15 00	2 75	34 21	23 98	3 85	2 204	1 511
Urine							1 315	00144	00101
Sheep 4									
Feces	37 85	24 41	14 57	2 48	33 86	24 68	3 91	2 304	1 662
Urine							1 384	00158	00101

There was a higher percentage of protein in the feces of the sheep when receiving grass dried at 400° F., indicating that they were not digesting this constituent so completely. There was a rather high percentage of nitrogen in the urine but the excretion of calcium and phosphorus from this source was very small.

APPARENT DIGESTIBILITY OF NUTRIENTS

The rations fed to the sheep were sufficient to meet their requirements for digestible nutrients as recommended in the Henry and Morrison standards (12). Due to the high percentage of protein in the grass the intake of that constituent was more than sufficient to meet the daily requirements of the wethers. As shown in table 4 the sheep made slight gains in live weight during each digestion trial except sheep no. 3 when receiving green grass. This animal did not adjust itself to the conditions of the experiment and therefore did not eat well. It was necessary to substitute another sheep for it in the later experiments.

TABLE 4.—Average weight of each sheep during the first 3 days and the last 3 days of the 21-day period on each of the rations

Ration	Average weight of—								
	Sheep no. 1—			Sheep no. 2—			Sheep no. 3—		
	Initial	Final	Gain	Initial	Final	Gain	Initial	Final	Gain
	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds
Green grass, no treatment	101 8	105 8	4 0	102 8	107 5	4 7	97 8	95 2	1 - 2 6
Sun-cured grass, exposed 18 hours	105 2	107 2	2 0	108 5	111 2	2 7			
Grass artificially dried at exhaust temperature of—									
250° F.	109 2	112 0	2 8	114 7	115 5	8	113 7	116 2	2 5
300° F.	113 0	113 8	8	117 7	120 0	2 3	115 5	119 5	4 0
350° F.	114 2	114 8	6	122 3	126 0	3 7	122 2	124 8	2 6
400° F.	115 3	117 3	2 0	125 3	129 2	3 9	125 0	127 8	2 8

¹ Loss.² Sheep no. 4 substituted for sheep no. 3, which went off feed in second trial

TABLE 5.—Dry matter and nutrients ingested, voided, and digested during a 14-day period by sheep receiving rations of green, sun-cured, and artificially dehydrated grass

GREEN GRASS, NO TREATMENT

Sheep no. and items compared		Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash
Sheep 1							
Fed	grams	19,540.0	3,196.7	4,193.3	766.0	8,986.4	2,397.6
Refused	do	817.2	148.7	184.7	27.5	350.3	105.9
Consumed	do	18,722.8	3,048.0	4,008.6	738.5	8,636.1	2,291.7
Voided	do	5,143.4	782.3	897.5	196.5	1,909.2	1,357.9
Digested	do	13,579.4	2,265.7	3,111.1	542.0	6,726.9	933.8
Digested	percent	72.5	74.3	77.6	73.4	77.9	40.7
Sheep 2							
Consumed	grams	20,130.0	3,293.3	4,319.9	789.1	9,257.2	70.0
Voided	do	6,517.3	985.4	1,237.0	289.4	2,377.5	1,628.0
Digested	do	13,612.7	2,307.9	3,082.9	499.7	6,880.3	842.0
Digested	percent	67.6	70.1	71.4	63.3	74.3	34.1
Sheep 3							
Fed	grams	19,180.0	3,134.6	4,111.7	751.1	8,811.7	2,350.9
Refused	do	2,897.0	509.0	677.6	81.4	1,241.6	387.3
Consumed	do	16,283.0	2,625.6	3,434.1	669.7	7,570.0	1,963.6
Voided	do	4,679.8	716.0	853.1	162.4	1,752.1	1,196.2
Digested	do	11,583.2	1,909.6	2,581.0	507.3	5,817.9	767.4
Digested	percent	71.2	72.7	75.2	75.8	76.8	39.1

SUN-CURED GRASS, EXPOSED 18 HOURS

Sheep 1							
Consumed	grams	15,482.9	2,913.9	3,180.2	535.7	6,908.5	1,944.6
Voided	do	3,710.4	631.5	492.4	193.3	1,203.6	1,189.5
Digested	do	11,772.5	2,282.4	2,687.8	342.4	5,704.9	755.1
Digested	percent	76.0	78.3	84.5	63.9	82.6	38.8
Sheep 2							
Consumed	grams	15,482.9	2,913.9	3,180.2	535.7	6,908.5	1,944.6
Voided	do	3,791.3	620.7	536.8	168.7	1,235.6	1,223.4
Digested	do	11,691.6	2,287.2	2,643.4	367.0	5,672.9	721.2
Digested	percent	76.5	78.5	83.1	68.5	82.1	37.1

TABLE 5.—Dry matter and nutrients ingested, voided, and digested during a 14-day period, by sheep receiving rations of green, sun-cured, and artificially dehydrated grass—Continued

GRASS ARTIFICIALLY DRIED AT 250° F.

Sheep no., and items compared		Dry matter	Crude protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash
Sheep 1:							
Consumed.....	grams	15,524 9	2,716 8	3,137 6	712 6	7,222 2	1,735 7
Voided.....	do	3,643 5	669 0	547 6	239 0	1,190 0	998 0
Digested.....	do	11,881 4	2,047 8	2,590 0	473 6	6,032 2	737 7
Digested.....	percent	76 5	75 4	82 6	66.5	83 5	42.5
Sheep 2:							
Consumed.....	grams	15,524 9	2,716 8	3,137 6	712 6	7,222 2	1,735 7
Voided.....	do	3,535 9	611 7	560 8	142 1	1,224 5	996 8
Digested.....	do	11,989 0	2,105 1	2,576 8	570 4	5,997 7	738 9
Digested.....	percent	77 2	77 5	82 1	80 1	83 0	42 6
Sheep 4:							
Consumed.....	grams	15,524 9	2,716 8	3,137 6	712 6	7,222 2	1,735 7
Voided.....	do	3,708 2	671 9	544 7	218 0	1,215 6	1,058 0
Digested.....	do	11,816 7	2,044 9	2,592 9	494 6	6,006 6	677 7
Digested.....	percent	76.1	75 3	82 6	69 4	83 2	39 0

GRASS ARTIFICIALLY DRIED AT 300° F.

Sheep 1:							
Consumed.....	grams	15,577 0	2,769 6	3,268 0	792 9	6,821 2	1,925 3
Voided.....	do	3,649 2	680 6	563 4	206 2	1,213 4	985 6
Digested.....	do	11,927 8	2,089 0	2,704 6	586 7	5,607 8	939 7
Digested.....	percent	76 6	75 4	82 8	74 0	82 2	48 8
Sheep 2:							
Consumed.....	grams	15,577 0	2,769 6	3,268 0	792 9	6,821 2	1,925 3
Voided.....	do	3,632 7	658 2	571 3	183 4	1,211 1	1,005 5
Digested.....	do	11,944 3	2,111 4	2,693 7	609 5	5,610 1	919 8
Digested.....	percent	76 7	76 2	82 1	76 9	82 2	47 8
Sheep 4:							
Consumed.....	grams	15,577 0	2,769 6	3,268 0	792 9	6,821 2	1,925 3
Voided.....	do	3,771 7	697 8	633 3	143 3	1,257 5	1,084 4
Digested.....	do	11,805 3	2,071 8	2,634 7	649 6	5,563 7	840 9
Digested.....	percent	75 8	74 8	80 6	81 9	81 6	43 7

GRASS ARTIFICIALLY DRIED AT 350° F.

Sheep 1:							
Consumed.....	grams	14,254 2	2,592 8	2,990 5	607 2	6,400 2	1,663 5
Voided.....	do	2,948 6	606 2	439 6	179 0	913 8	777 0
Digested.....	do	11,305 6	1,986 6	2,550 9	428 2	5,486 5	886 5
Digested.....	percent	79.3	76 5	85 3	70 5	85 2	53 3
Sheep 2:							
Consumed.....	grams	15,550 1	2,828 6	3,262 4	662 4	6,982 0	1,814 7
Voided.....	do	3,497 0	604 9	523 2	140 9	1,205 1	933 0
Digested.....	do	12,053 1	2,133 7	2,739 2	521 5	5,776 9	881 7
Digested.....	percent	77 5	75 4	84 0	78 7	82 7	48 6
Sheep 4:							
Consumed.....	grams	15,550 1	2,828 6	3,262 4	662 4	6,982 0	1,814 7
Voided.....	do	3,495 5	712 4	515 6	129 3	1,190 6	947 6
Digested.....	do	12,054 6	2,116 2	2,746 8	533 1	5,791 4	867 1
Digested.....	percent	77 5	74 8	84 2	80 5	83 0	47 8

GRASS ARTIFICIALLY DRIED AT 400° F.

Sheep 1:							
Consumed.....	grams	14,023 6	2,521 4	3,208 6	706 8	5,954.4	1,632 3
Voided.....	do	4,241 9	1,057 5	599 4	169 7	1,383.7	1,031 6
Digested.....	do	9,781 7	1,463 9	2,609 2	537 1	4,570.7	600 7
Digested.....	percent	69.8	58.1	81 3	76 0	76.8	36 8
Sheep 2:							
Consumed.....	grams	15,328 1	2,756.0	3,507.1	772 5	6,508 3	1,784.2
Voided.....	do	4,627 6	1,113 4	694 2	127 3	1,583.1	1,109 7
Digested.....	do	10,700 5	1,642 6	2,812.9	645.2	4,925 2	674.5
Digested.....	percent	69.8	59 6	80 2	83 5	75.7	37 8
Sheep 4:							
Consumed.....	grams	15,328 1	2,756.0	3,507 1	772 5	6,508.3	1,784.2
Voided.....	do	4,545 4	1,109 5	662 3	112 7	1,539.1	1,121 8
Digested.....	do	10,782 7	1,646.5	2,844 8	656.8	4,968.2	662 4
Digested.....	percent	70 3	59.7	81.1	85 4	76 4	37 1

Table 5 contains data on the dry matter and nutrients ingested, voided, and digested by sheep receiving the different rations. The digestibility coefficients of the individual wethers in each trial are in close agreement for all constituents except the ether extract, which varies considerably.

At the beginning of the experiment with the sun-cured grass, sheep no. 3 went off feed for several days and it was necessary to reject results from it in summarizing the results of the trial.

Table 6 shows the apparent digestibility of the different rations.

TABLE 6.—*Apparent digestibility of green, sun-cured, and artificially dehydrated grass*

Ration	Sheep	Dry matter	Crude protein	Crude fiber	Ether extract	Nitro- gen-free extract	Ash
	Number	Percent	Percent	Percent	Percent	Percent	Percent
Green grass, no treatment	3	70.4	72.3	74.6	70.5	76.3	37.8
Sun-cured grass, exposed 18 hours	2	75.8	78.4	83.8	66.2	82.4	38.0
Grass artificially dried at exhaust temperature of							
250° F.	3	76.6	76.0	82.4	72.0	83.2	41.4
300° F.	3	76.4	75.5	81.9	77.6	82.0	46.8
350° F.	3	78.1	75.6	84.5	76.8	83.6	49.8
400° F.	3	70.0	59.2	80.9	81.8	76.2	37.3

The digestibility for the various constituents of the sun-cured grass and of that dried at 250°, 300°, and 350° F. showed no significant variation. The digestibility of the constituents in these rations was high, the average being 76 percent for dry matter, 76 for crude protein, 83 for crude fiber, 73 for ether extract, 83 for nitrogen-free extract, and 44 for ash. The sun-cured grass was as efficiently digested as the grass dried by artificial means.

When the temperature of drying was increased to a high level (i. e., the exhaust-gas temperature being 400° F.) for the purpose of securing maximum evaporation of moisture, a lowering of the digestibility of certain of the feed constituents resulted. The apparent digestibility of the constituents of pasture herbage dried at 250°, 300°, and 350° was as high as similar grass dried by 18 hours of exposure in the sunlight. The apparent digestibility of the crude protein of the grass dried at 400° was depressed since it was only 78.3 percent of that of the grass dried at 350°. The apparent digestibility of the dry matter and nitrogen-free extract was also somewhat depressed, being practically the same as the green grass. These findings are in accord with results reported by Watson and Ferguson (18) who noted a depression of the digestibility of the protein of grass at an outlet temperature of 200° C. (392° F.).

The apparent digestibility of the various nutrients when the sheep were receiving the green grass was not so high as when they were receiving the dried material. The reasons for this are not entirely apparent. The consumption of dry matter when the sheep were receiving green grass was approximately 15 percent greater than when they were on the dried-grass ration, which may account for a lower utilization of feed nutrients.

NITROGEN, CALCIUM, AND PHOSPHORUS BALANCES

The data pertaining to the nitrogen, calcium, and phosphorus balances of sheep receiving the rations of green, sun-cured, and artificially dehydrated grass are given in table 7. The balance of nitrogen in each case was positive, indicating that the wethers were assimilating considerable of that constituent. Except in the case of the grass dried at 400° F., there was from 2½ to 3 times as much nitrogen recovered in the urine as in the feces indicating that the protein was digested, absorbed, and a large part of the nitrogen was eliminated.

TABLE 7.—Grams of nitrogen, calcium, and phosphorus ingested, voided, and retained by sheep during 14-day metabolism trials with different rations

GREEN GRASS, NO TREATMENT

Sheep no	Nitrogen					Calcium					Phosphorus				
	Ingested	Voided			Retained	Ingested	Voided			Retained	Ingested	Voided			Retained
		Feces	Urine	Total			Feces	Urine	Total			Feces	Urine	Total	
Sheep 1	490.5	125.0	308.1	433.1	57.4	142.5	123.2	3.6	126.8	15.7	90.6	72.6	0.4	72.9	17.7
Sheep 2	527.4	158.4	326.8	485.2	42.3	153.2	155.6	1.6	157.3	14.1	97.4	89.4	4.4	89.7	7.7
Sheep 3	426.1	114.6	293.6	408.3	27.8	123.8	112.8	2.2	115.6	8.2	78.7	67.1	2.6	67.4	11.4
Average	478.0	132.7	306.2	438.8	39.2	139.8	130.5	2.7	133.2	6.6	88.9	76.3	3.0	76.7	12.3

SUN-CURED GRASS, EXPOSED 18 HOURS

Sheep 1	466.0	100.9	297.8	398.7	67.3	113.3	99.2	0.2	99.5	13.8	100.2	74.6	0.2	74.8	25.4
Sheep 2	466.0	100.1	308.1	408.2	57.8	113.3	102.3	1.0	103.3	10.0	100.2	74.7	2.2	75.0	25.2
Average	466.0	100.5	302.9	403.4	62.6	113.3	100.8	6.0	101.4	12.0	100.2	74.4	2.2	71.9	25.3

GRASS ARTIFICIALLY DRIED AT 250° F

Sheep 1	434.7	107.1	271.0	378.1	56.6	113.2	96.2	0.6	97.4	15.8	80.1	67.6	0.2	67.8	12.3
Sheep 2	434.7	97.9	387.1	385.0	49.6	113.2	98.6	4.4	99.0	14.2	80.1	67.6	2.2	67.8	12.3
Sheep 4	434.7	107.5	293.8	371.4	63.4	113.2	100.1	5.5	100.6	12.6	80.1	71.1	2.2	71.6	8.5
Average	434.7	104.2	271.0	378.2	56.5	113.2	98.5	5.5	99.0	14.2	80.1	68.9	2.2	69.1	11.0

GRASS ARTIFICIALLY DRIED AT 300° F.

Sheep 1	442.4	109.0	265.4	374.2	68.2	118.7	98.7	0.4	99.0	19.7	86.4	68.7	0.2	68.7	17.6
Sheep 2	442.4	105.4	278.4	384.7	57.7	118.7	99.9	4.4	100.1	18.6	86.4	66.7	2.2	66.8	19.6
Sheep 4	442.4	111.6	275.0	386.6	55.8	118.7	100.1	3.0	100.4	18.3	86.4	72.3	2.2	72.5	13.9
Average	442.4	108.6	272.9	371.5	60.9	118.7	99.5	4.4	99.9	18.8	86.4	69.2	2.2	69.4	17.0

GRASS ARTIFICIALLY DRIED AT 350° F

Sheep 1	414.8	97.6	228.7	326.3	88.5	95.5	74.8	0.3	75.1	20.4	73.7	51.6	0.1	51.7	22.0
Sheep 2	452.5	111.2	253.9	365.1	87.4	104.2	90.0	.6	90.6	13.6	80.4	61.8	.1	62.1	18.2
Sheep 3	452.5	114.0	263.4	377.3	75.2	104.2	91.5	.4	92.0	12.2	80.4	63.1	.1	63.2	17.2
Average	439.9	107.6	248.6	356.2	83.8	101.3	85.5	4.4	85.1	15.4	78.2	58.8	.1	59.0	19.2

GRASS ARTIFICIALLY DRIED AT 400° F.

Sheep 1	403.9	169.2	171.8	341.1	62.8	96.6	93.0	0.2	93.2	3.4	81.8	66.7	0.2	66.8	14.9
Sheep 2	441.4	178.2	202.9	381.1	60.4	105.6	102.0	.2	102.2	3.4	89.4	71.3	.2	71.5	17.9
Sheep 4	441.4	177.7	218.6	396.3	45.1	105.6	104.7	.2	105.0	.6	89.4	75.5	.2	75.7	13.7
Average	428.9	175.0	197.8	372.8	56.1	102.6	99.9	.2	100.1	2.5	86.8	71.2	.2	71.3	15.5

¹ This was a minus quantity.

The percentage of nitrogen retained was small and varied considerably with the feeding of the different grass rations. The lowest retention of nitrogen occurred in the experiment with green grass, the average being about 8 percent. The nitrogen recovered in the feces (table 7) was somewhat higher during this trial than on subsequent experiments except for the grass dried at 400° F. where there was a marked depression of the digestibility of the protein. The highest retention of nitrogen occurred when the sheep were receiving the grass dehydrated at 350°. During this trial 19 percent of the ingested nitrogen was retained. There was little variation in the nitrogen balances of sheep when on the rations of sun-cured grass as compared with dehydrated grass dried at either 250°, 300°, or 400°.

The sheep maintained positive calcium balances during each trial, with the exception of sheep no. 2 when it was receiving green grass. This animal failed to utilize any of the calcium in the green grass. There were decided variations in the utilization of calcium by the three sheep during this experiment. This variation may be accounted for by the fact that this was the first trial and the sheep had been on an unknown diet in the stockyard for some time prior to these studies. Such variations were not encountered in subsequent trials. The average calcium balances were not significantly different when the sheep received rations of sun-cured grass and dehydrated grass dried at 250°, 300°, and 350° F.

The sheep receiving rations of pasture grass dried at 400° F. did not utilize the calcium of the rations as efficiently as when receiving similar herbage dried at lower temperatures or cured in the sun. The average calcium balance for sheep receiving rations of grass dried at 400° F. was +2.5 as compared with a balance of +15.4 when receiving grass dried at 350° and a balance of +12.0 when receiving the sun-cured grass. The average calcium balance for the grass dried at 400° was only 16.2 percent as great as for the grass dried at 350° and 20.8 percent as great as for the sun-cured grass.

There was considerably more phosphorus than calcium retained by the sheep when receiving the green grass. The phosphorus balance was highest when the wethers were receiving the sun-cured grass. Apparently the condition causing the poor utilization of calcium in the grass dried at 400° F. did not affect the availability of the phosphorus. The utilization of phosphorus from this grass was approximately as high as from the other dehydrated samples.

The average daily intake of calcium on the various rations was as follows: Green grass, 10.0 g; sun-cured grass, 8.1 g; artificially dehydrated grass dried at 250° F., 8.1 g; dried at 300°, 8.4 g; at 350°, 7.2 g; and at 400°, 7.3 g. The average daily consumption of phosphorus by the sheep on the rations listed was 6.4, 7.2, 5.7, 6.2, 5.6, and 6.2 g, respectively. According to Fraps (8) this amount of calcium and phosphorus is considerably in excess of the needs of the wethers used in this experiment. He states that in order for sheep weighing about 100 pounds to maintain a mineral balance they should receive approximately 2 g calcium and 0.54 g phosphorus in their daily ration. While there is some variation in the calcium and phosphorus content of the various rations, this difference does not seem great enough to cause the wide variation in retention of these constituents. The retention of calcium and phosphorus does not seem to bear any relation to the calcium-phosphorus ratio of the rations. The retention of calcium

and phosphorus during the first two trials, which included rations of green and sun-cured grass, may have been influenced by the previous unknown diet of the sheep. While 14-day mineral-balance trials do not give as definite results as longer trials, it does not seem probable that possible deficiencies in pre-experimental rations could have carried through to the trial with the grass dried at 400° F. which followed 16 weeks of feeding on similar rations of high calcium or phosphorus content. It would seem, therefore, that artificial drying of the grass at 400° definitely lowered the calcium retention.

COLOR DETERMINATIONS

A comparison of the color of the different rations of the dried pasture grass is given in table 8. Conversion factors for measuring the percentage of natural color of pasture herbage have not been developed and it is therefore necessary to use those established for alfalfa hay to make the comparison. It is thought that the conversion factors for alfalfa are somewhat high for pasture herbage. The percentage of natural color was highest in the grass that was dried in the sun. This sample of grass was spread thinly on a canvas and exposed to the sunlight. It was taken in at night so was not wet by either rain or dew. This indicates that exposure to moisture is probably a more important factor in causing loss of color than exposure to light. It is probable that grass cured under these conditions would show a higher degree of natural color than ordinary field-cured hay. There were only slight differences between the percentage of natural color of the sun-cured grass and that of grass dried at 250° and 300° F. The color of the dehydrated samples, however, might have been higher had they been dried immediately after cutting, rather than allowed to wilt overnight. The percentage of natural color of the grass dried at 400° was only 76.3 percent of that dried at 250°. The importance of providing forage of a high natural color is indicated by Graves (9) who reports a relation between the green color and the assimilable carotene of the feed as well as the yellow color of butterfat produced from the feed.

TABLE 8.—*Relation of temperature of drying to the percentage of natural color of artificially dried pasture grass*

Treatment of grass	Average Munsell hue reading	Percentage of natural color	Grade in terms of alfalfa
Sun-cured grass, exposed on canvas 18 hours	9 50	78	Extra green
Grass artificially dried at exhaust temperature of			
250° F	9 22	76	Do
300° F	8.81	74	Grade 1
350° F	7.28	65	Do.
400° F	6 15	58	Grade 2.

SUMMARY AND CONCLUSIONS

Six digestive and mineral-balance trials were conducted to determine the effect of the temperature of artificial drying on the apparent digestibility and availability of the feed nutrients in 3-week-old mixed pasture grass. The grass mixture contained about 40 percent of English ryegrass, 40 percent of Italian ryegrass, and 20 percent of white

clover. Exclusive rations of green grass, sun-cured grass, and artificially dehydrated grass dried at exhaust-gas temperatures of 250°, 300°, 350°, and 400° F. were fed to yearling wethers for periods of 21 days, the first 7 of which were preliminary periods.

The percentage of apparent digestibility of the feed constituents in the rations of sun-cured grass and artificially dehydrated grass dried at 250°, 300°, and 350° F. showed no significant differences, indicating that artificial drying at these temperatures had no deleterious effect upon the availability of the nutrients. The percentage of apparent digestibility of the nutrients in the rations of sun-cured grass and dehydrated grass dried at 250°, 300°, and 350° F. averaged for dry matter, 76; for crude protein, 76; for crude fiber, 83; for ether extract, 73; for nitrogen-free extract, 83; and for ash, 44.

The results of feeding pasture grass artificially dehydrated at 400° F. showed that this temperature of drying had a depressing effect on the apparent digestibility of the crude protein, dry matter, and nitrogen-free extract. There was some evidence of burning of the more leafy portions of the herbage when dehydrated at 400° F.

The apparent digestibility of dry matter, crude protein, crude fiber, and nitrogen-free extract was slightly lower for the green grass than for the dried samples, with the exception of that dried at 400° F.

Positive balances of nitrogen, calcium, and phosphorus were maintained by the sheep receiving the six different rations. There was no apparent relationship between the average nitrogen balance and the temperature of artificial drying.

The average calcium balance for the grass dried at 400° F. was very low as compared with the balance for the other dried samples, since it was only 16 percent of the balance obtained for the grass dried at 350° F. The factor causing the low calcium balance for the grass dried at 400° did not act accordingly on the phosphorus.

Under the conditions of this experiment, the percentage of natural color of the herbage was highest in the sample cured in the sun. As the exhaust-gas temperature was increased by 50-degree intervals from 250° to 400° F. in drying the different samples there was a definite lowering of the percentage of natural color in the herbage. The percentage of natural color of the herbage dried at an exhaust-gas temperature of 400° was only 76.3 percent of that dried at 250°.

From the standpoint of the feeding value of the dried product, it is apparent that artificial drying at 400° F. is an uneconomical practice with the machine used in this experiment. Probably the most efficient temperature at which to operate this machine is from 300° to 350°. If high temperatures are employed in the commercial drier without running the temperature at the exhaust end as high as those found to be detrimental in this experiment, forage may probably be dried with little or no depressing effect on the digestibility of the nutrients. Undoubtedly the digestibility of the nutrients in herbage is also influenced by the length of time during which it is subjected to relatively high temperatures.

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THE THERMOPHILIC FERMENTATION OF BEET PULP¹

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INTRODUCTION

The mechanisms of the dissimilative processes involved in the thermophilic fermentation of complex substances such as beet pulp are not clear, but for practical purposes the fermentation may be considered essentially as the break-down of the various carbohydrate constituents, i. e., pectin, pentosans, cellulose, and residual sugars. The importance of the bacterial decomposition of cellulose has been pointed out by Lymn and Langwell (14)², Tetrault (15), Coolhaas (2), Fred, Peterson, and coworkers (9, 12, 16), and others. The products of this fermentation are acetic and butyric acids, ethyl alcohol, carbon dioxide, hydrogen, and methane. Lymn and Langwell report wide variations in the yields as a result of manipulative treatment of the cultures. Of almost equal importance, not only industrially but also from an agricultural standpoint, is the fermentation of the hemicelluloses and, of somewhat less importance, of the pectins, both of which occur widely distributed in agricultural wastes. Although the thermophilic fermentation of cellulose has been studied for many years, that of the other important constituents of plant tissues for the most part has been neglected.

The present work on the thermophilic fermentation of beet pulp was undertaken with a view to effecting the utilization of an important agricultural byproduct. The important products of this fermentation are acetic and butyric acids, but hydrogen and carbon dioxide are also formed.

EXPERIMENTAL

MATERIALS AND CULTURES

Two kinds of beet pulp were used in the fermentations. One was the dried cossettes left after the extraction of the sugar at the factory; the other was obtained by adding waste molasses to the cossettes and drying (table 1).

TABLE 1.—Percentage composition of beet pulps used

Material	Pento- sans	Pectin	Cellu- lose	Total sugar	Lignin	Ether extract	Crude protein	Ash	Mois- ture
Commercial beet pulp	16 0	34 0	18 0	3.2	2 5	3 5	9 6	2 7	7 5
Beet pulp plus molasses	11 0	26 5	12 5	17 0	2.2	1 1	19.9	5.4	5 3

Mixed bacterial cultures were obtained from sod-manure compost, garden soil, and stable and sheep manure. Enrichment cultures were

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² Reference is made by number (italic) to Literature Cited, p. 172.

made by inoculating small quantities of beet-pulp broth and incubating anaerobically at 60° C. for about 48 hours. All fermentations were carried out anaerobically at 60°. In the later stages of the work the only cultures used were enrichment cultures from garden soil, since the products yielded were practically the same in all cases.

BACTERIOLOGICAL STUDIES

The bacteriological study of the fermentation of plant tissues offers many points of interest and many difficulties. It seemed a remote possibility that the complete fermentation of a complex substance such as beet pulp would be brought about by a single bacterial species. It was soon found impracticable, however, to attempt to use pure cultures. After a few transfers had been made the fermentations became sluggish. Purified pectin in concentrations of 0.5 to 2 percent was used in media which were inoculated with crude cultures and fermented in anaerobic jars. Ammonium chloride was used as a source of nitrogen in some cases; peptone was used in others. A fairly active fermentation resulted in all cases. Similar media were prepared, and adjusted to pH 7.0, and transfers were made from the original fermentations. Varying quantities of the fermenting broth were used as inocula. These fermentations were less vigorous. Repeated transfers led to sluggish fermentations. The same experience was encountered in the fermentation of purified xylan. The second transfer brought about a very slow fermentation.

For the isolation of colonies a beet-pulp yeast-infusion agar was used. About 100 g of beet pulp and 50 g of dried yeast in 1 l of water were heated for 15 minutes in the autoclave. After cooling, the supernatant liquid was decanted. Two grams of dipotassium phosphate and 15 g of agar were added, and the medium was again sterilized in the autoclave. The medium was always heated in the autoclave just before being used. Plates were prepared from suitable dilutions of actively fermenting cultures. These plates were placed in vacuum desiccators with actively fermenting broth in the bottom. The desiccators were evacuated and filled with carbon dioxide, and the process was repeated several times. The evolution of gas from the growing cultures in the bottoms of the desiccators maintained a positive carbon dioxide pressure and effectively prevented the diffusion of air into the vessels. Smooth, translucent colonies grew in 24 to 48 hours.

The colonies were transplanted into flasks of beet-pulp broth made from beet pulp containing 17 percent sugar. In a few of the flasks a fairly active fermentation occurred. Transfers were made from these flasks to sugar-free beet pulp, but the fermentations were very slow and unsatisfactory. None of the colonies isolated was regarded as a pure culture; consequently the cultural characteristics were not determined.

The repeated transfer of cultures from one beet-pulp broth to another had the effect of lessening the vigor of fermentation. More formic acid was found in solution after a few transfers were made, but no definite relationship could be established. The effect of the transfer of cultures is indicated in table 2. The building up of an active culture for this fermentation is of considerable importance and should be given further study, especially for the decomposition of pentosans.

TABLE 2.—*Effect of transferring culture on thermophilic fermentation of beet pulp*¹

Times transferred	Total acid produced ²		Formic acid	Times transferred	Total acid produced ²		Formic acid
	Grams	Percent	Percent		Grams	Percent	Percent
-----	21	52.5	0.0	5	9	22.5	5.0
-----	18	45.0	1.0	5	14	35.0	7.5
-----	15	37.5	10.0	7	8	20.0	2.0

¹ 40 grams of beet pulp in each sample² Calculated as acetic acid.

While the bacteriological aspects of this work are of scientific and practical importance, it was necessary for the time being to set them aside and continue with the more practical use of crude cultures for the utilization of various forms of agricultural wastes.

ANALYTICAL METHODS

Proximate analyses of the beet pulp were made for the constituents shown in table 1. The official current methods of the Association of Official Agricultural Chemists (1) were used for the determination of sugars, pentosans, crude ether extracts, protein, moisture, and ash. Cellulose was determined by the method of Mehta (6). Pectin was extracted from the pulp with 0.5 percent ammonium oxalate, according to the method of Schryver and Haynes (11), and determined by the procedure recommended by Nanji, Paton, and Ling (7). Lignin was determined by Schorger's method (10).

According to Schryver and Haynes (11), pectin yields 19.8 percent of furfural. Corrections were made accordingly in the determination of the pentosans.

After the fermentations were complete, the liquors were filtered, and analyses were made on both the liquids and solid residues to determine the unaltered constituents. It is possible that the pectin may not have been completely fermented, yet partly hydrolyzed or altered in such a manner as to escape detection after fermentation. The routine examinations for pectin were made after the fermentation.

The volatile acids produced were steam-distilled from an aliquot of the fermentation liquor which had been adjusted to an approximate pH of 4 with sulphuric acid. The acids were determined by the partition method, as described by Osburn and Werkman (8).

Carbon dioxide and hydrogen were determined by collecting the gas over water and analyzing the gas mixture in the usual manner. Various forms of apparatus for the collection of gas were made and studied. For thermophilic work the simple expedient mentioned above was the most efficient.

FERMENTATION STUDIES

OXYGEN REQUIREMENTS

Three series of experiments were set up in order to study the oxygen requirements of the organisms. (1) The quantities of beet pulp given in table 3 were added to 1,500 cc of water. Five grams of ammonium chloride and two grams of dipotassium phosphate were added. After inoculation the flasks were aerated for the time indicated. (2) The flasks were stoppered loosely with cotton and shaken

several times a day. (3) In the third series, various kinds of anaerobic apparatus were constructed and used, in some of which elaborate precautions were taken to exclude air. Time and convenience considered, the most efficient was a flask of suitable size with a rubber stopper carrying 2 delivery tubes, 1 of which dipped into mercury. After the medium was inoculated, oxygen-free nitrogen was blown through the flask, and the second delivery tube was sealed. The media were boiled immediately before inoculation to remove air. The data in table 3 indicate that strictly anaerobic conditions result in more complete decomposition of the beet pulp.

TABLE 3.—*Effect of oxygen tension on the thermophilic fermentation of beet pulp*¹

AERATED CULTURE				
Duration of experiment (days)	Total acid produced ²	Acids present		
		Acetic	Propionic	Butyric
	Grams	Percent	Percent	Percent
20	1 40	(¹)	(¹)	(¹)
10	1 10	(²)	(²)	(¹)
COTTON-STOPPERED FLASK				
10	12 56	86 0	0	11 0
8	10 37	81 5	0	18 5
15	14 75	79 5	0	20 5
ANAEROBIC CULTURE				
10	13 14	57 5	33 8	8 5
10	13 90	60 8	23 0	7 2
10	16 20	80 8	0	19 6
10	16 10	70 7	0	29 3

¹ 40 grams of beet pulp in each sample² Calculated as acetic³ Not determined

INFLUENCE OF pH

Anaerobic fermentations were conducted at pH levels of 5.4, 7.2, and 9.0, as nearly as they could be controlled. For the fermentation at pH 5.4, chlorphenol red was used as an indicator. The broth was neutralized as required. The indicator mixtures for the other two fermentations were prepared according to the method of Kolthoff and Menzel (5). At pH 7.2 a mixture of 1 part of 0.1-percent bromthymol blue and 1 part of 0.1-percent phenol red was used. The color changed from yellow to green at pH 7.2 and finally to deep violet at 7.6. Owing to the coloration of the medium, the color changes were sometimes difficult to observe, especially if iron salts were present. At pH 9, 1 part of 0.1-percent thymol blue and 3 parts of 0.1-percent phenolphthalein were used. The broth in all cases was neutralized with sodium hydroxide as required.

The data are given in tables 4 and 5. Complete analyses were made before and after fermentation. The periods of fermentation ranged from 4 to 8 days. It is noteworthy that no hydrogen was produced at pH 9.0 and that while the volatile acids were nearly doubled, as compared with those in the fermentation at 5.4, the grams of carbon

dioxide remained nearly constant (table 5). Very little is known concerning the mechanism of the fermentation of complex substances. Free sugars could never be detected, nor could any intermediate compounds be isolated. The products of fermentation account for 78 percent (at pH 5.4) to 85 percent (at pH 9.0) of the carbon fermented. Ehrlich's (3) formula for beet pectin was used in making the calculations. According to the method of Johnson, Peterson, and Fred (4) for calculating the oxidation-reduction balance of the products of fermentation, there is an excess of oxidized products in each fermentation. At pH 9.0 the oxidation products are much in excess (-0.70 to $+0.25$). The residue of unfermented beet pulp, including the protein and possibly some degradation products of pectin, is far too complex to permit conjecture as to any reduction products unaccounted for, or the nonappearance of hydrogen at pH 9.0.

TABLE 4 --Results of thermophilic fermentation of beet-pulp constituents at different pH levels

Constituent	pH 5.4 (100.5 g)			pH 7.2 (101.4 g)			pH 9.0 (102.5 g)		
	Original sample		Fermented	Original sample		Fermented	Original sample		Fermented
	Grams	Grams		Grams	Grams		Grams	Grams	
Total sugars	17.1	17.1	100.0	17.2	17.2	100.0	17.4	17.4	100.0
Pectin	26.6	20.8	78.2	26.9	26.9	100.0	27.3	27.3	100.0
Pentosans	11.1	8.6	77.5	11.2	8.9	79.4	11.3	9.7	85.8
Celldulose	12.6	2.1	16.6	12.7	1.3	10.2	12.8	7.2	56.2
Lignin	2.2	0	0	2.2	0	0	2.3	0	0

TABLE 5 --Products of thermophilic fermentation of beet pulp at different pH levels

Product	pH 5.4 (100.5 g)	pH 7.2 (101.4 g)	pH 9.0 (102.5 g)
	Grams	Grams	Grams
Hydrogen	0.37	0.23	(¹)
Carbon dioxide	14.70	14.90	15.60
Total acid	27.30	34.21	42.60
Formic	(²)	11	60
Acetic	13.20	16.70	26.40
Propionic	6.60	9.20	12.10
Butyric	7.50	8.20	3.50

¹ None

² Trace

EFFICIENCY OF NEUTRALIZING AGENTS

Calcium carbonate, sodium bicarbonate, and sodium hydroxide were used as neutralizing agents. The quantities of sugar-free pulp shown in table 6 were inoculated with soil-enrichment cultures. An excess of calcium carbonate was added to some of the flasks. Enough sodium bicarbonate to neutralize acetic acid equivalent to half the weight of the sample of beet pulp used was added to each of the flasks used in experiment 2, and the third set was neutralized from time to time with sodium hydroxide. The results given in table 6 indicate that sodium bicarbonate is the most efficient neutralizing agent used. Sodium carbonate would be used in large-scale operations. Sodium hydroxide would probably be equally efficient if the pH value could be more closely controlled. The inefficiency of calcium carbonate is probably

due to the low pH. The pH remained between 4 and 5 during the experiment. Stirring was purposely avoided.

TABLE 6.—*Efficiency of various neutralizing agents as evidenced by acid production in the fermentation of beet pulp*¹

Neutralizing agent	Acid produced ²		Neutralizing agent	Acid produced ²	
	Grams	Percent		Grams	Percent
CaCO ₃	6 0	15 0	NaHCO ₃	15 8	39 5
CaCO ₃	6.2	15 5	NaOH.....	10 0	25 0
NaHCO ₃	16 0	40 0	NaOH.....	11.5	28 7

¹ 40 grams of sugar-free beet pulp in each sample

² Calculated as acetic

FERMENTATION OF THE DIFFERENT CONSTITUENTS OF BEET PULP

A series of fermentations was conducted to determine further the optimum conditions. Complete proximate analyses were made after fermentation in order to determine the unfermented components of the pulp. Thirty grams of sugar-free beet pulp, 5 g of ammonium chloride, and 2 g of dipotassium phosphate were added to 1,500 cc of water in 2-l flasks. The flasks were inoculated with enrichment cultures from soil. The grams of each component in the sample and the percentage of each component fermented are given in table 7. The yields and compositions of the acids are given in table 8.

TABLE 7.—*Fermentation of beet-pulp constituents under different experimental conditions*^{1 2}

Constituent	Original sample	Fermented in—					
		Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6
		Grams	Percent	Percent	Percent	Percent	Percent
Pentosans.....	4 62	87 0	87 5	70 0	96 0	86 5	78 0
Pectin.....	10 02	58 5	75 0	62 0	58 0	74 0	59 0
Total sugars.....	70	100 0	100 0	100 0	100 0	100 0	100 0
Cellulose.....	5 37	82 5	84 5	6 5	88 0	89 0	30 4
Lignin.....	67	0	0	0	0	0	0

¹ 30 grams of pulp in each sample

² Hydrogen gas was passed through flasks 1, 2, and 3. 15 g of sodium bicarbonate was added to flasks 1 and 2 at the time of inoculation, flask 3 was neutralized with sodium hydroxide (to phenolphthalein) as required. Carbon dioxide was passed through flasks 4, 5, and 6. 15 g of sodium bicarbonate was added to flasks 4 and 5; flask 6 was neutralized with sodium hydroxide as required

TABLE 8.—*Acids produced by fermentation of beet-pulp constituents under the different conditions shown in table 7*

Flask no.	Total acid produced	Acids present ¹			Flask no	Total acid produced	Acids present ¹		
		Formic	Acetic	Butyric			Formic	Acetic	Butyric
		Grams	Percent	Percent			Percent	Percent	Percent
.....	11 1	0	86	14	4.....	13.1	0	85	15
.....	12.0	0	76	24	5.....	14.7	0	86	14
.....	7.1	27	60	13	6.....	7 5	0	85	15

¹ No propionic acid produced

The greatest effect is to be noted in the fermentation of cellulose. In the presence of the sodium bicarbonate, upwards of 80 percent of the cellulose was fermented; whereas in the other flasks only small percentages were fermented. It is possible that a strain of specific cellulose-fermenting organisms is present in the crude cultures and that conditions for their growth in flasks 3 and 6 were unfavorable. A pure culture of cellulose-fermenting organisms did not ferment beet pulp. The pure culture of Snieszko (13), Peterson, and Fred,³ while active in a cellulose medium, failed entirely to ferment beet pulp.

Lignin was not altered during the period of the fermentation. Apparently pectin is somewhat resistant to fermentation, only about 75 percent breaking down under the most favorable conditions.

Pure cellulose is readily fermented when it is added to beet pulp and inoculated with crude cultures from the soil. Ten grams of beet pulp and five grams of filter paper in 800 cc of water were inoculated with 25 cc of a soil-enrichment culture. The cellulose was entirely fermented at the end of 7 days. The yield of acids was equivalent to 60 percent of the combined weight of pulp and filter paper. The possibilities of the utilization of mixed substrates are being further investigated in this laboratory.

Fermentations conducted under optimum conditions as determined by previous experiments gave yields of acids ranging from 35 to 53 percent of the weight of the beet pulp. The higher yields were obtained from the pulp containing about 17 percent total sugars. Typical results are given in table 9.

TABLE 9 -Results of thermophilic fermentation of beet pulp with soil-enrichment cultures

Pulp in sample (grams)	Total acid produced ¹		Acids present		
			Formic	Acetic	Butyric
	Gram.	Percent	Percent	Percent	Percent
500	267	53.4	0.0	91.0	9.0
500	268	53.6	.0	91.0	9.0
500	250	50.0	0	89.5	10.5
50	26	52.0	3.0	86.5	10.5
40 ²	18	45.0	2.0	85.0	13.0
40 ²	16	40.0	0	81.5	18.5
40 ²	14	35.0	.0	85.0	15.0

¹ Calculated as acetic acid

² Sugar-free beet pulp.

SUMMARY

The thermophilic fermentation of beet pulp by soil-enrichment cultures has been found to be most efficient at pH 9.0 under strictly anaerobic conditions.

Acetic and butyric acids, and occasionally propionic acid, were formed. The maximum yield was 53 percent of the weight of the beet pulp.

The cellulose, pectin, and pentosans of beet pulp were fermented at about the same rates. Lignin was unaltered.

Repeated transfers of the crude cultures led to sluggish fermentation.

³ Kindly furnished by Dr. Fred.

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THE ISOLATION AND DISTRIBUTION OF NITROGEN IN DILUTE ALKALI-SOLUBLE PROTEINS OF HEALTHY VALENCIA AND WASHINGTON NAVEL ORANGE FRUITS¹

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INTRODUCTION

It is generally recognized that the application of nitrogenous fertilizers to the soil has an important bearing on the metabolism of the citrus tree and on the production of normal fruit, and yet the percentage of total nitrogen in the mature fruit is very low. The effect of nitrogenous fertilization on the accumulation of nitrogen in the tissues has not been determined for citrus as it has been for certain other plants.

This paper presents the results of one phase of a general investigation of the nitrogenous constituents of citrus fruits. The investigation includes a study of the effects of fumigation, sprays, storage, and other factors on these constituents. The present paper deals only with the isolation and distribution of nitrogen in the proteins of the solids in the edible portion of healthy orange fruits.

REVIEW OF LITERATURE

Very few papers have been published bearing on the organic forms of nitrogen existing in *Citrus*, and practically no correlation has been made of their function in metabolism.

Smith (13)² isolated a protein from the edible portion of the orange and very briefly described its general properties. It was found to be soluble in dilute alkali (0.3 percent) and insoluble in water, neutral salts, and weak acids. It was not coagulated by heat in neutral or alkaline solution and it possessed an isoelectric point of pH 4.7.

Nelson, Mottern, and Eddy (9) identified nitrogenous compounds such as arginine, asparagine, choline, and stachydrine, and a mixture of monoamino acids in the juice of the Valencia orange.

Saunders (12) and Rotha and Saunders (11) treated orange-seed meal with various alkali halides in normal concentrations and observed that the various salts extracted the same amounts of nitrogen. They also found that the protein isolated by each of these different salts had the same amount of nitrogen. As a result of their work they concluded that they were dealing with a definite globulin and that this was the only type of protein existing in the seed of the orange.

The proteins which have been found in the edible portion of the orange and which are characterized by being insoluble in neutral solvents but soluble in dilute acids and alkalis (5), are not commonly found in fleshy, acid fruits. They usually occur in the endosperms of

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² Reference is made by number (italic) to Literature Cited, p. 180.

seeds, as found by Larmour (8), and may exist in the cytoplasm of the green tissues, as found by Chibnall (3), who isolated such a protein from spinach leaves.

EXPERIMENTAL METHODS

ISOLATION OF THE PROTEIN

The method used in extracting the protein from the Valencia orange fruit was a modification of the method used by Smith (13). It was as follows:

Pulp of burred oranges or separator pulp was washed with cold water. "Separator pulp" is the term applied to the suspended solids which have been thrown down by centrifuging the mechanically macerated, fleshy part of the fruit. The washed pulp was then extracted with an equal volume of 95-percent alcohol containing 1-percent NaOH. This material was filtered through cotton cloth. The pulp was discarded and an equal volume of water was added to the filtrate. Acetic acid was added slowly to the filtrate to bring it to pH 4.7, at which point the protein settled out as a flocculent precipitate. The precipitate was washed with distilled water until acid-free. It was then extracted with alcohol (95 percent) to remove color, and finally washed with absolute alcohol and anhydrous ether and dried in a vacuum oven at 40° C.

The protein was then placed in a 0.3-percent NaOH solution and filtered. Much of this material would not dissolve in 0.3-percent alkali. The insoluble residue was treated with 0.3-percent alkali until solution of the protein ceased. The protein was then precipitated at pH 4.7 with acetic acid. The processes of dissolving and precipitating were repeated three times. Finally the precipitated protein was washed with 95-percent alcohol, absolute alcohol, and anhydrous ether, and was dried in a vacuum oven at 40° C. The protein was then in the form of a friable, grayish-white powder.

A more elaborate procedure was required for the extraction of protein from the Washington navel orange. Complications arose owing to the possible existence of two proteins in the pulp. The protein material also appeared to be more firmly associated with the chromophores than in the Valencia orange. The method employed for isolating protein material from the Washington navel orange was as follows:

The pulp was washed with hot water. The washed pulp was extracted three times with 95-percent ethyl alcohol to free the material of pigments. The pulp was then dried in vacuum and subsequently extracted with 5-percent NaOH. The alkali extract was treated with 2 volumes of 95-percent ethyl alcohol and filtered. The protein was precipitated from the filtrate with acetic acid at pH 4.6 to 4.7. After the material had been washed free of acid with distilled water, it was shaken with 95-percent alcohol containing 1-percent NaOH. Some of the precipitated protein was dissolved in this solution and was designated as protein A, while that which remained insoluble in the alcoholic alkali was designated as protein B.

The solution containing protein A was precipitated at pH 4.6 to 4.7 with acetic acid and filtered. The acid was removed from the filtrate by washing with distilled water, the color was removed by using 95-

percent ethyl alcohol, and, finally, the precipitate was washed with anhydrous ether and dried in a vacuum oven at 40° C.

Preliminary tests demonstrated that this product was contaminated with carbohydrates. Further purification was accomplished by dissolving it in a 0.3-percent NaOH solution, filtering, and precipitating the protein by adding dilute acetic acid until the solution reached a pH value of 4.6 to 4.7. The protein was dehydrated with 95-percent ethyl alcohol, absolute alcohol, anhydrous ether, and finally dried in vacuum at 40° C. This procedure was performed a second time. When the protein was precipitated at pH 4.7 and allowed to stand overnight, the supernatant liquid was free from any turbidity.

The insoluble material designated as the protein B fraction contained much carbohydrate material and a gummy substance of a colloidal nature. Distilled water was added to this material, which was already alkaline, until the protein dissolved. The protein was then precipitated at pH 4.7 with acetic acid. After the precipitate had been filtered, the protein was extracted with a 1-percent acetic acid solution until washings were free of carbohydrate. It was next freed of acid with distilled water, and of color with 95-percent ethyl alcohol, and finally washed with anhydrous ethyl ether, and dried in vacuum at 40° C. The total nitrogen of this material was 12.29 percent, corrected for moisture and ash, the latter being 0.95 percent. Since at this time it was impossible to obtain enough material for further experimentation, no attempt was made to determine whether this nitrogen-containing material was similar to the protein already isolated or to another protein having different physical and chemical properties.

CHEMICAL METHODS

The nitrogen fractions recorded in table 1 were determined by slight modifications of the methods of Van Slyke (14). The apparatus used in the ammonia nitrogen determination was the same as that described by Cavett (2). The latter procedure saved much time and yet retained the accuracy of the original method. The modified method employed by Plimmer and Rosedale (10) was used in some of the determinations. According to this method the phosphotungstic acid was removed from the basic filtrate as well as from the basic fraction by the addition of BaCl₂. This was done to rid the filtrate of salts that crystallized during the distillation process, and it allowed the filtrate to be reduced to a much smaller known volume. It was found, however, that phosphotungstic acid did not interfere with the amino nitrogen determination of the filtrate. In the amino nitrogen determination, it was desirable that the final volume of the basic filtrate should be as small as possible, since 2-cc aliquots were taken for the determination.

The apparatus used in the arginine determination was that described by Holm (7). When samples of protein weighing less than 1 g were used, the modified apparatus of Cavett (2) was found very satisfactory. In the determination of the ammonia fraction, the distillate was received in N/14 H₂SO₄, while in the other nitrogen determinations, the ammonia was received in 4-percent H₃BO₃.

TABLE 1.—*Nitrogen distribution of protein of the Valencia and Washington navel orange pulp, expressed as percentage of the total nitrogen*

Nitrogen containing compound or form of nitrogen	Valencia			Washington navel		
	A	B	Average	A	B	Average
Ammonia.....	5 05	5 38	5 21	7 15	6 87	7 01
Acid insoluble	1 35	1 30	1 33	80	77	79
Acid soluble	1 48	1 40	1 44	88	77	82
Total humin..	2 83	2 70	2 77	1 68	1 54	1 61
Arginine.....	12 14	12 28	12 21	12 55	12 66	12 61
Histidine.....	2 55	2 64	2 60	2 58	2 47	2 52
Cystine.....						
Lysine.....	10 73	10 78	10 75	10 48	10 40	10 44
Total basic	25 42	25 70	25 56	25 61	25 53	25 57
Amino.....	58 50	58 91	58 70	58 75	59 25	59 00
Nonamino.....	7 87	7 46	7 67	7 38	7 24	7 31
Total nonbasic	66 37	66 37	66 37	66 13	66 49	66 31
Total nitrogen	99 67	100 15	99 91	100 57	100 43	100 50

The modification of Larmour (8) was used in the precipitation of the basic fraction. Instead of the solution being heated for 1 hour after the bases had been precipitated with phosphotungstic acid, the two separate solutions, that is, the basic and the phosphotungstic acid solutions, were heated to boiling and poured together.

THE ANALYSIS OF THE PROTEINS

The percentage of total nitrogen of the proteins from the two kinds of oranges are recorded in table 2, corrected for moisture and ash. The figures for the ash content of both proteins are not all that could be desired, but to free the protein of all of its ash constituents would require many precipitations which would result in great loss of protein. The proteins were precipitated at pH 4.6 to 4.7, as shown in the table. They also had a minimum solubility at these pH values when precipitated from a solution of 0.3-percent NaOH by the addition of dilute acetic acid.

TABLE 2.—*Comparative analyses (percentage) of Valencia and Washington navel orange protein*

Constituents	Valencia	Washington navel	Constituents	Valencia	Washington navel
Nitrogen.....	15 12	15 37	Ash.....	1 05	0 70
Moisture.....	7 32	8 70	pH.....	4 76	4 60-4 70

† Isoelectrically precipitated

The data recorded in table 1 show the distribution of nitrogen in the proteins from the Valencia and Washington navel orange pulp, respectively. The proteins of the Valencia orange possessed more humin nitrogen than the protein of the Washington navel orange, which is probably due to contamination with carbohydrates which

furnish an adequate amount of aldehyde for humin formation. The acid hydrolysates of these proteins were dark in color, which is characteristic of hydrolysates high in humin nitrogen.

The ammonia nitrogen was somewhat higher in the navel orange protein. This value of a protein analysis is supposedly derived from the amide nitrogen of the monoamino dicarboxylic acids and also from deamination of some of the α -amino groups. Gortner and Holm (6) have shown that the ammonia derived from the deamination of the amino groups varies with the length of hydrolysis. They found that on prolonged hydrolysis appreciable deamination of the amino groups may occur, giving rise to an abnormally high value for the true amide nitrogen.

The values of the basic nitrogen of the two proteins are the same, within the limits of experimental error. This value represents the nitrogen precipitated with phosphotungstic acid, and is a measure of the arginine, cystine, histidine, and lysine content. The calculations of the percentage of these amino acids were made according to the method of Van Slyke (14).

The nonbasic nitrogen which represents the monoamino monocarboxylic acids and monoamino dicarboxylic acids is 66 percent of the total nitrogen. More than 58 percent of the total nitrogen occurs in the monobasic fraction in the amino form, while more than 7 percent occurs as nonamino nitrogen.

An effort was made to determine quantitatively the amount of pentose in the protein material for the purpose of ascertaining whether the pentose is precipitated with the protein as an impurity or whether it is an integral part of the protein molecule. This was done by determining the yield of furfural from 0.5 g of orange protein distilled according to the official method (1). The distilled furfural (or methyl furfural) was subsequently precipitated with phloroglucinol. The amount of furfural obtained was 4.7 mg, which, in all probability, was derived from the arabinose and galacturonic acid of pectin. The decarboxylation of galacturonic acid in the presence of the 12-percent HCl yields arabinose which, in turn, would produce furfural. Hence, it is very probable, as Smith (13) has noted, that the pentose reaction was derived from the pectin accompanying the protein in the precipitation process and that it is not an integral part of the protein molecule. It should be mentioned here that the phloroglucide precipitate was nearly all soluble in 95-percent ethyl alcohol. It may be that the presence of this alcohol-soluble phloroglucide (4) was due to the formation of methyl furfural from the hydrolysis of pectin.

It is known that when pectin is treated with dilute NaOH, a soluble sodium pectate is produced, and when the pectate is acidified with HCl a gelatinous, insoluble pectic acid is formed. This is probably the reason why it is so difficult to free the protein of pentoses, or furfural-yielding substances, by repeated precipitation from dilute NaOH, for during the process the pectic acid is also partially precipitated.

A previous investigation (13) has shown that this protein gives the biuret reaction, yielding the pink color characteristic of proteose and peptone. The alkaline solution of orange protein possessed no turbidity and therefore showed no Tyndal cone. When a 1-percent solution of the protein, dissolved in 0.3-percent NaOH, was placed

in a cellophane bag and dialyzed against distilled water for 48 hours, no protein diffused through the bag. Some of the sodium came through the membrane, but enough was retained in the bag to prevent the precipitation of the protein during the 48-hour dialysis.

Experiments were performed to determine the effect of heat on the precipitation of the protein from a 0.3-percent NaOH solution. Fifty cubic centimeters of a 1-percent navel orange protein solution was brought to the boiling point in 4 minutes and maintained at a boiling temperature for 5 minutes, cooled to room temperature, and then precipitated at pH 4.7 by the addition of acetic acid. These experiments were repeated except that the boiling time was lengthened to 10 and 20 minutes, respectively. In each case a noticeable difference occurred in the rate of precipitation from the alkaline solution. In the case of the solutions boiled for 20 minutes, some of the protein was precipitated at pH 4.7, but much of it remained in dispersion and the supernatant liquid did not clear as it had done in the control experiments. This demonstrated that the very short period of heating of the protein solution at boiling temperature in dilute alkali produced enough hydrolysis or cleavage in the molecule to alter its precipitation at the isoelectric point.

DISCUSSION

Table 3 records the amide, humin, basic, and nonbasic nitrogen of proteins soluble in dilute alkali from various sources, as reported by other investigators and the writers. An inspection of table 3 shows a similarity between the nitrogen fractions of the proteins of Valencia and navel orange fruits and those of other plant tissues. It is not claimed however that all proteins soluble in dilute alkali would yield nitrogen fractions similar to those reported in the table, for existing evidence (8) shows otherwise. It is sufficient to say that the values demonstrate the existence of a class of proteins in plant tissues, soluble in dilute alkali, which is characterized by a relatively high content of both basic nitrogen (25 percent \pm) and of amide nitrogen (5 to 10 percent).

TABLE 3.—Comparison of the amide, humin, basic, and nonbasic nitrogen of the proteins soluble in dilute alkali from various plant sources

Nitrogen	Percentage of protein from indicated sources					
	Spinach leaves ¹	Rice ²	Rye ²	Spelt ²	Valencia orange fruit	Washington navel orange fruit
Amide.....	6.94	8.07	7.14	8.06	5.21	7.01
Humin.....	2.06	1.95	4.30	5.02	2.77	1.61
Basic.....	26.59	27.59	25.79	24.44	25.56	25.57
Nonbasic.....	64.41	62.52	62.50	62.07	66.37	66.31

¹ Data from Chibnall (3).

² Data from Larmour (8).

As Smith (13) has noted, the proteins of the orange exist in the solid state. This is manifested by the fact that these proteins are soluble in dilute alkali and are precipitated at pH 4.7, while the pH of the juice is approximately 3.5.

Further research is necessary to establish the factors affecting the equilibrium which in all probability must exist between the proteins in the solid state in the fruit and other simpler nitrogenous compounds which are known to exist in solution. A previous investigation (9) has identified in the juice of Valencia oranges such soluble nitrogenous compounds as arginine, asparagine, choline, and stachydrine, and a mixture of monoamino acids. It is very probable that the amino acids are synthesized in the leaves, transported to the fruit, and later synthesized to protein in the fruit. When the nitrogen bases are considered, little is known of their direct function in plant metabolism, and it has never been determined whether these compounds are directly active in protein synthesis or are only metabolic products formed by the breaking down of amino acids in the plant.

SUMMARY AND CONCLUSIONS

Detailed methods have been described for the isolation and purification of the dilute alkali-soluble proteins from the Valencia and Washington navel orange pulp, respectively. The distribution of the nitrogen of these proteins has been determined according to the method of Van Slyke (14), as modified by various other workers.

The experimental results recorded in this paper appear to warrant the following conclusions:

The protein from the Valencia orange pulp has a nitrogen content of 15.12 percent and that from the Washington navel orange pulp, 15.37 percent, both being corrected for moisture and ash.

The basic amino acid fraction and the nonbasic fraction of the two proteins are practically the same.

Protein from the Washington navel orange has a slightly higher percentage of ammonia nitrogen than that from the Valencia orange, but the protein from the latter has a higher percentage of humin nitrogen. It is possible that impurities may account for the differences in the humin values.

Both of these proteins were precipitated in pH 4.6 to 4.7. This is the pH range of minimum solubility as determined by the amount of protein remaining in solution after precipitating the protein at these pH values.

After repeated precipitation from a 0.3-percent NaOH solution, the protein contained carbohydrate material. When the protein was distilled with 12-percent HCl, furfural was produced, which in turn yielded an alcohol-soluble phloroglucide when precipitated with phloroglucinol.

When a dilute alkali solution (0.3-percent NaOH) of Washington navel orange protein was heated to the boiling temperature for 5, 10, and 20 minutes, respectively, a difference occurred in the rate of precipitation of the protein at its isoelectric point. Heat did not coagulate the protein.

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THE VITAMIN A CONTENT OF FIVE VARIETIES OF SWEETPOTATO¹

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INTRODUCTION

Steenbock and Sell (9)² in 1922 studied three varieties of sweetpotatoes and reported that the amount of vitamin A found in them varied with the depth of color. More recent investigations (1, 3, 5) have indicated clearly that vitamin A activity varies with the carotene content of plant products. In 1931 Rice and Munsell (6) reported the vitamin A content of sweetpotatoes tested by them to be 1,360 Sherman units per pound.

The experiments described in this paper were carried out for the purpose of determining the vitamin A value of different types of sweetpotato (*Ipomoea batatas* (L.) Lam.) commonly grown in Tennessee. Three of the varieties studied—the Nancy Hall, Yellow Jersey, and Porto Rico—are strongly pigmented, the Porto Rico having the deepest color and the Nancy Hall and Yellow Jersey having about the same depth of color (4). The Triumph and Southern Queen are much lighter in color. The sweetpotatoes were grown on the University of Tennessee Farm. They were cured at 85° to 90° F. and stored at 50° to 60°.

The Sherman and Munsell (8) method of testing for vitamin A as modified by Sherman and Burtis (7) was used in all of the experiments.

The potato was fed raw and was cut in such a way that the animal always obtained a representative cross-section of it.

VITAMIN A CONTENT OF THE NANCY HALL SWEETPOTATO

The studies dealing with the vitamin A value of the Nancy Hall sweetpotato were carried out during 1 year.

The first series of experiments was started immediately after the harvesting of the sweetpotatoes. More variable results were obtained and a lower vitamin A value was found in these experiments than in tests carried out several months after harvesting. The feeding of the sweetpotato was continued from early in November through June.

Figure 1 shows the results of the experiments in which the Nancy Hall variety was fed. When 0.025 g was fed daily to each animal 6 days in the week, 8 animals lost an average of 6 g in 8 weeks. When 0.05 g was fed daily, 10 animals made an average gain of 57 g in 8 weeks. Therefore, the quantity of this variety which contains 1 vitamin A unit, that is, the amount which will induce a gain of 25 g in 8 weeks in a standard test rat, is between 0.025 and 0.05 g, or approximately 0.035 g. The Nancy Hall variety contains, therefore, about 30 units of vitamin A per gram, or 13,500 units per pound.

¹ Received for publication Dec. 21, 1934; issued April, 1935. Contribution from the Agricultural Experiment Station and the School of Home Economics of the University of Tennessee.

² Reference is made by number (*italic*) to Literature Cited, p. 186

VITAMIN A CONTENT OF THE PORTO RICO SWEETPOTATO

The Porto Rico variety was studied at two different times during the year. In series 1 the sweetpotatoes were fed directly after harvesting, while in series 2 they were fed after having been stored for 2 months or more. The results of the experiments of the two series varied too much to be averaged together, and they are therefore presented separately, as series 1 and series 2.

SERIES 1

The results of series 1, in which the Porto Rico sweetpotato was fed directly after harvesting, are shown in figure 2, A. The gains made by the different animals receiving the same amount were variable. The results indicated, however, that a unit of vitamin A was contained in somewhat less than 0.05 g of the Porto Rico variety. Four animals each of which received 0.05 g of sweetpotato per day gained an average of 34 g in the 8 weeks of the experimental period, while 4 which received 0.025 g each per day lost an average of 15 g. These results indicate that at this season, directly after harvesting, the Porto Rico variety contained about 20 units of vitamin A per gram, or 9,000 units per pound.

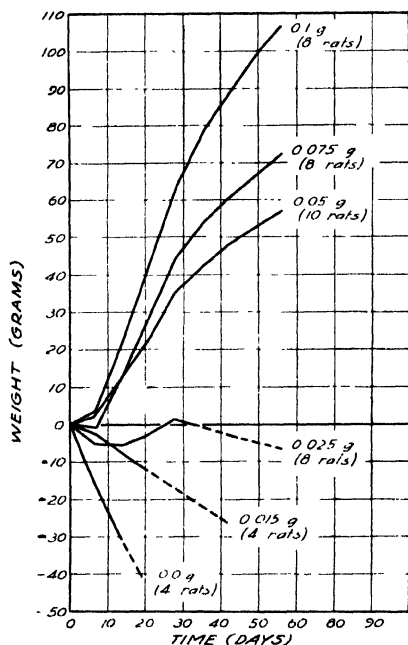


FIGURE 1. - Vitamin A content of the Nancy Hall sweetpotato, showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve; broken lines indicate the death of one or more animals

SERIES 2

The results of the experiments of series 2, in which the Porto Rico variety was fed 2 months or more after harvesting, are given in figure 2, B. Seven rats each of which received 0.015 g of sweetpotato per day made an average gain of 37 g in an 8-week period. This was approximately the same gain as that made by the animals of series 1, which

received 0.05 g per day. The vitamin A value of the potato had increased about three times, therefore, in the few months after harvesting. Amounts varying from 0.0075 g per rat per day to 0.075 g were fed in this series. The results indicate that the Porto Rico sweetpotato, after storage for a few months, contained at least 65 units of vitamin A per gram, or 29,000 per pound. Fraps and Treichler (2) reported 50 units per gram, as the vitamin A value of Porto Rico sweetpotatoes, a figure which compares favorably with that found in series 2.

The marked increase in the vitamin A value of the sweetpotatoes which was observed during the year could not be accounted for by

changed conditions in the laboratory or by any change in the animals. The negative control animals used in the experiments of series 1 lived 23.4 days and lost an average of 40.3 g, while those of series 2 lived 25.0 days and lost an average of 36.0 g. These findings clearly show that the animals of the two series reacted similarly to a diet devoid of vitamin A, and prove that any differences in the results of the two series were due to changes taking place in the sweetpotato during storage.

VITAMIN A CONTENT OF THE YELLOW JERSEY SWEETPOTATO

The Yellow Jersey sweetpotato used in this experiment had a deep yellow flesh, similar in depth of color to the Nancy Hall variety.

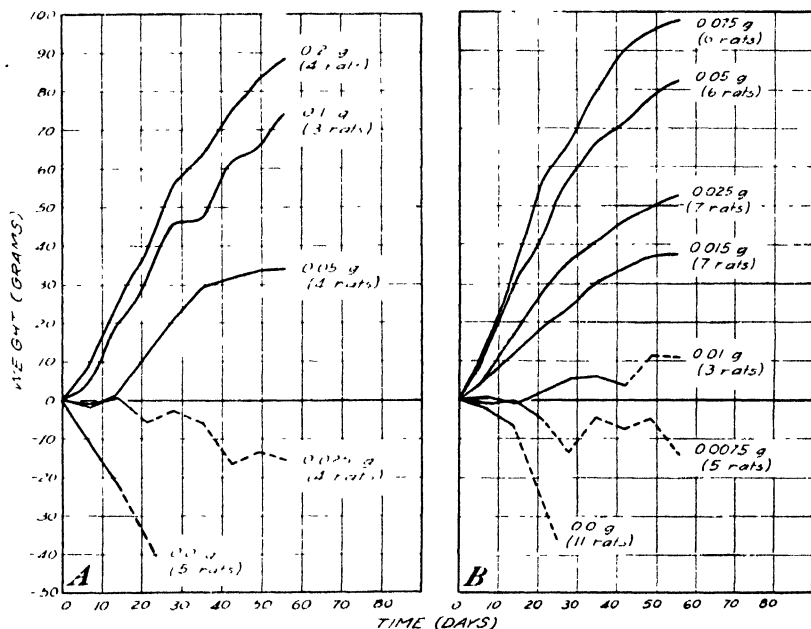


FIGURE 2—Vitamin A content of the Porto Rico sweetpotato in experiments of series 1 (A) and series 2 (B), showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve, broken lines indicate the death of one or more animals. In series 1 the sweetpotatoes were fed directly after harvesting, in series 2 they were fed several months after harvesting.

The studies reported here were made at two different times of the year and during the same year in which the experiments on the Porto Rico variety were carried out. The experiments of series 1 were performed in the fall, directly after harvesting, and those of series 2 several months later.

SERIES 1

Amounts of Yellow Jersey sweetpotato varying from 0.025 g to 0.2 g per rat per day were fed directly after harvesting. The results are shown in figure 3, A. Four rats receiving 0.1 g each per day made an average gain of 10 g in 8 weeks, while three rats receiving 0.2 g each per day made an average gain of 80 g. These results indicate that the Yellow Jersey variety directly after harvest contained less

than 1 vitamin A unit in 0.1 g; that is, less than 10 units per gram or less than 4,500 units per pound.

SERIES 2

In a second series of experiments Yellow Jersey sweetpotato was fed after it had been in storage for several months. The amounts fed varied from 0.015 to 0.1 g per rat per day. The results are shown in figure 3, *B*. When 0.025 g per rat per day was fed to 7 rats, the animals made an average gain of 25 g in the 8 weeks of the experimen-

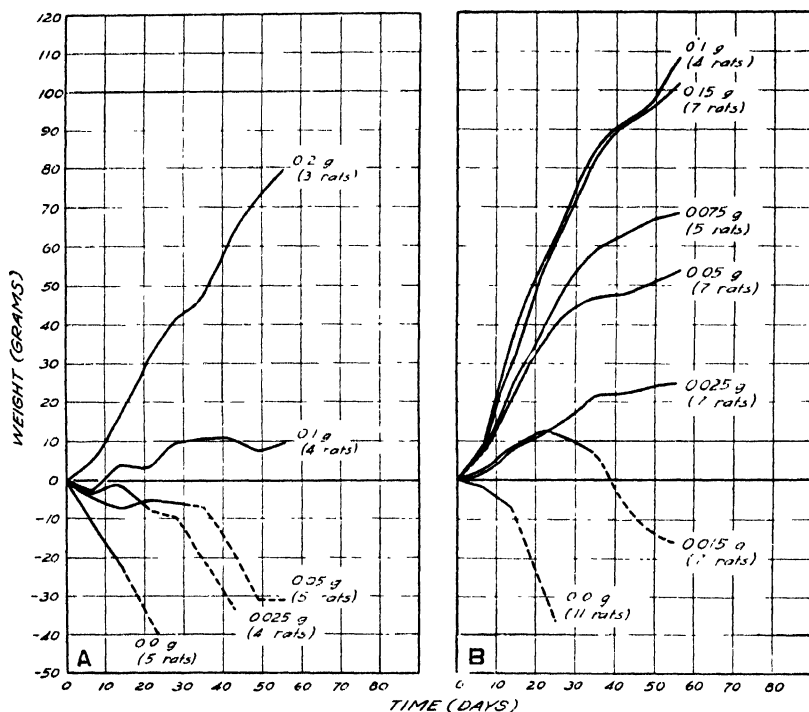


FIGURE 3.—Vitamin A content of the Yellow Jersey sweetpotato in experiments of series 1 (*A*) and series 2 (*B*), showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve, broken lines indicate the death of one or more animals. In series 1 the sweetpotatoes were fed directly after harvesting, in series 2 they were fed several months after harvesting.

tal period. When 0.05 g per rat per day was fed to 7 rats, the animals made an average gain of 53 g. The sweetpotato at this time, therefore, contained about 40 units of vitamin A per gram, or 18,000 units per pound. The vitamin A potency of the sweetpotato had increased at least four times during storage.

It is apparent, therefore, that a change takes place in the vitamin A content of sweetpotatoes during storage. Determinations of the water content of different varieties of sweetpotato at different times of the year³ have shown that the moisture content does not change sufficiently to account for the variation in vitamin A activity. The increase in vitamin A activity in the months after harvesting may

³ Unpublished data. University of Tennessee

indicate that the carotene in the sweetpotato is not fully developed when the roots are first harvested, that the carotene has not yet developed into the precursor of vitamin A, or else that this precursor (the carotene responsible for the formation of vitamin A *in vivo*) is present in a form less available to the animal body when the sweetpotatoes are first harvested than after they have been stored for some time.

VITAMIN A CONTENT OF THE TRIUMPH SWEETPOTATO

The Triumph sweetpotato was first fed directly after harvesting. The levels of feeding chosen, which were the same as those used in testing the Nancy Hall, Yellow Jersey, and Porto Rico varieties, were too small to promote growth. All the animals in these tests died before the end of the experimental period and showed typical symptoms of vitamin A deficiency.

Another group of animals were fed after the potatoes had been stored for 2 months or more. Feeding levels of 0.2, 0.4, 0.6, and 0.8 g per rat per day 6 days per week were given. Eight rats receiving 0.2 g per rat per day lost an average of 9.8 g in the 8 weeks experimental period. Eleven rats receiving 0.4 g per rat made an average gain of 21.2 g, while 3 rats which received 0.6 g per day made an average gain of 45.3 g. The Triumph variety contains, therefore, about 2 units of vitamin A per gram, or 900 units per pound. The results are shown in figure 4.

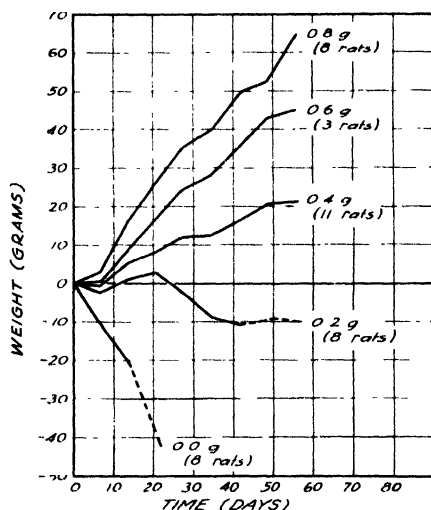


FIGURE 4. Vitamin A content of the Triumph sweetpotato, showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve. Broken lines indicate the death of one or more animals.

VITAMIN A CONTENT OF THE SOUTHERN QUEEN SWEETPOTATO

The Southern Queen was also found to be a poorer source of vitamin A than the Nancy Hall, Porto Rico, and Yellow Jersey varieties. The same levels of feeding were used in testing this variety as in the tests with the Triumph variety. It was fed only after storage for 2 months or more. Ten rats which received 0.2 g of the Southern Queen variety per rat per day made an average gain of 21.7 g in the 8 weeks period, while 11 which received 0.4 g made an average gain of 52.3 g.

The Southern Queen variety contained, therefore, about 4 vitamin A units per gram, or 1,800 units per pound. The results are shown in figure 5.

SUMMARY AND CONCLUSIONS

The vitamin A content of five varieties of sweetpotato—Nancy Hall, Porto Rico, Yellow Jersey, Triumph, and Southern Queen was studied.

The Porto Rico and Yellow Jersey types were investigated at two different seasons; first, directly after harvesting in the fall, and again 2 or more months after harvesting. The Nancy Hall, Triumph, and Southern Queen varieties were tested only after they had been stored for some time.

The Nancy Hall variety was found to contain 30 units of vitamin A per gram, or 13,500 units per pound; the Triumph, 2 units per gram, or 900 units per pound; and the Southern Queen, 4 units per gram, or 1,800 units per pound.

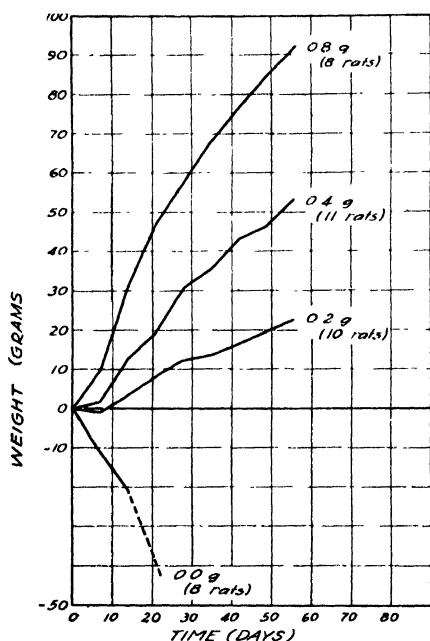


FIGURE 5—Vitamin A content of the Southern Queen sweetpotato, showing average gain or loss in weight of rats fed this sweetpotato as their sole source of vitamin A. The amount of sweetpotato fed per rat per day and the number of rats in the group are shown at the end of each curve, broken lines indicate the death of one or more animals.

The Porto Rico variety was found to contain about 20 units of vitamin A per gram, or 9,000 units per pound, directly after harvesting. After the potatoes had been stored for 2 months or more, the usual period of storage before consumption, they contained about 65 units per gram, or 29,000 units per pound. The vitamin A value of these sweetpotatoes had increased, therefore, slightly more than three times.

Directly after harvesting, the Yellow Jersey sweetpotato contained less than 10 units per gram, or less than 4,500 units per pound. After storage for 2 months or more, the sweetpotatoes contained 40 units per gram, or 18,000 units per pound. The vitamin A value had increased in this case about four times.

Possible reasons for the increase in vitamin A during storage are given.

The Nancy Hall, Porto Rico, and Yellow Jersey varieties were found to be much richer sources of vitamin A than the Triumph and Southern Queen varieties.^t Vitamin A values were found to vary with depth of color.

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No. 3

HISTOLOGICAL CHARACTERISTICS OF PLANTS GROWN IN TOXIC CONCENTRATIONS OF BORON¹

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INTRODUCTION

Although small amounts of boron are considered essential for the normal growth and development of many widely different species of the higher green plants (7, 8, 15, 16, 17),² excessive concentrations of this element have long been known to be toxic (11). Injury resulting from boron occurring in excessive concentrations as a natural constituent of irrigation waters is a factor of agricultural importance in certain areas in the western United States (5, 9, 13). The wide variability of optimum and maximum concentrations of boron for the growth of different species and the characteristic external symptoms of boron injury exhibited by numerous plants have been discussed recently by Eaton (5). Generally, leaf injury is the characteristic manifestation of boron toxicity, the older leaves showing the most pronounced symptoms. Briefly, the common symptoms of boron injury to leaves are the yellowing of tips and margins (often followed by marginal or spotted browning, which may be followed by premature defoliation) and the subsequent production of malformed leaves. Under high-boron conditions stone-fruit trees seldom exhibit these leaf symptoms, but the petioles and larger veins of the leaves of prune and apricot trees may become brown and rough and occasionally exude gum. The twigs frequently exude gum, especially above the leaf and twig insertions, and die at the tips; their internodes may be much shortened, and their nodes are often enlarged.

MATERIAL AND METHODS

The histology of plant organs showing boron injury has hitherto received but scant attention. The specimens upon which the present study is based were mostly taken from the cultures of this Division at the Rubidoux Laboratory, Riverside, Calif. The boron-injured prune specimens were from a plant grown in a sand culture supplied with a culture solution containing 9 parts per million of boron. The boron-injured peach, apricot, and grape were supplied with a solution containing 6 p. p. m. of boron; and the boron-injured lemon was supplied with a solution containing 3 p. p. m. of boron. These specimens were studied in comparison with healthy specimens of the same age and variety grown under similar conditions except for lower concentrations of boron in the culture solution. In the case of lemon and grape, the healthy specimens received 1 p. p. m. of boron in the culture solution. Specimens of healthy peach, prune, and apricot

¹ Received for publication Nov. 12, 1934; issued April 1935.

² Reference is made by number (italic) to Literature Cited, p. 193.

were grown in a culture solution to which no boron had been added. The boron contained in these solutions was generally less than 0.1 p. p. m., occurring as impurities in the c. p. chemicals or in the quartz sand. Specimens of field-grown prunes and apricots supplied with irrigation water of high boron content and comparable specimens from adjacent orchards irrigated with water of low boron content were also examined. The material was sectioned fresh or after killing and fixing in formalin-acetic-alcohol, and mounted unstained in glycerin.

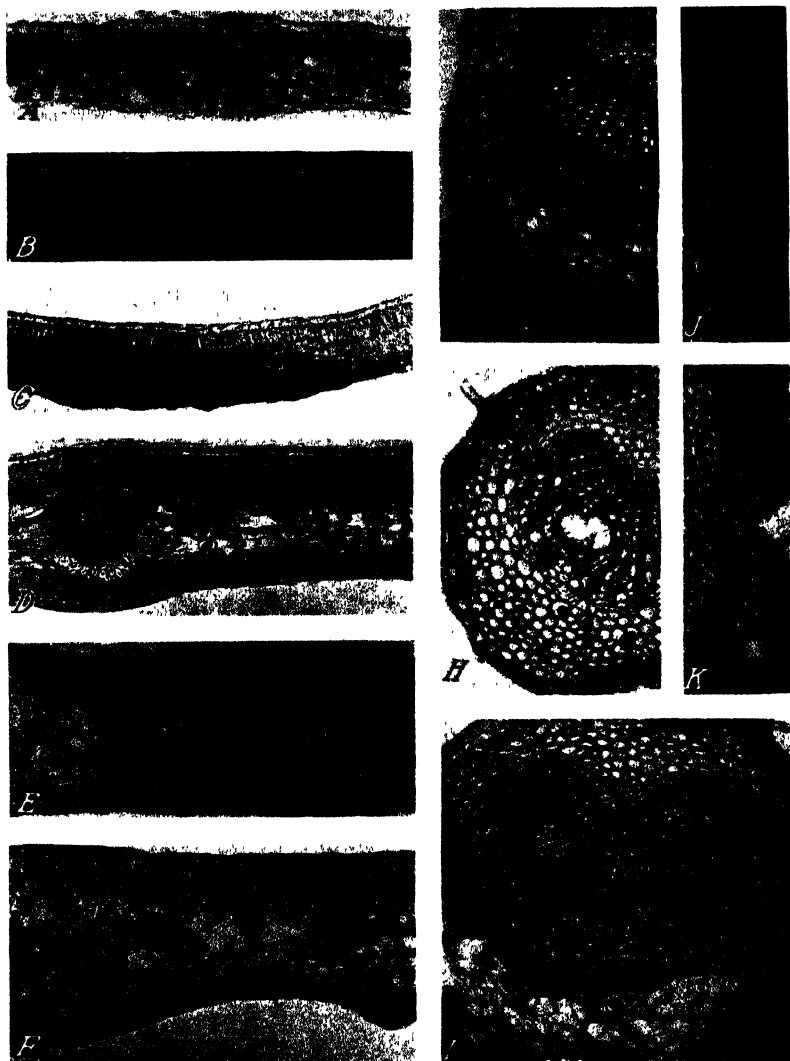
ABNORMALITIES OF LEAVES

A histological study of boron-injured leaves of lemon, grape, prune, and apricot showed that their structural abnormalities occur chiefly at points showing macroscopically visible injury. The yellowish areas which commonly precede the brown margins of boron-injured lemon leaves and the pale-green areas which often border the brown spots of boron-injured grape leaves are attributable to the conversion of chloroplasts into leucoplasts in such areas. Except for the reduced number of chloroplasts, the mesophyll of injured leaves shows no histological differences from that of healthy leaves at these points (pl. 1, *A-F*).

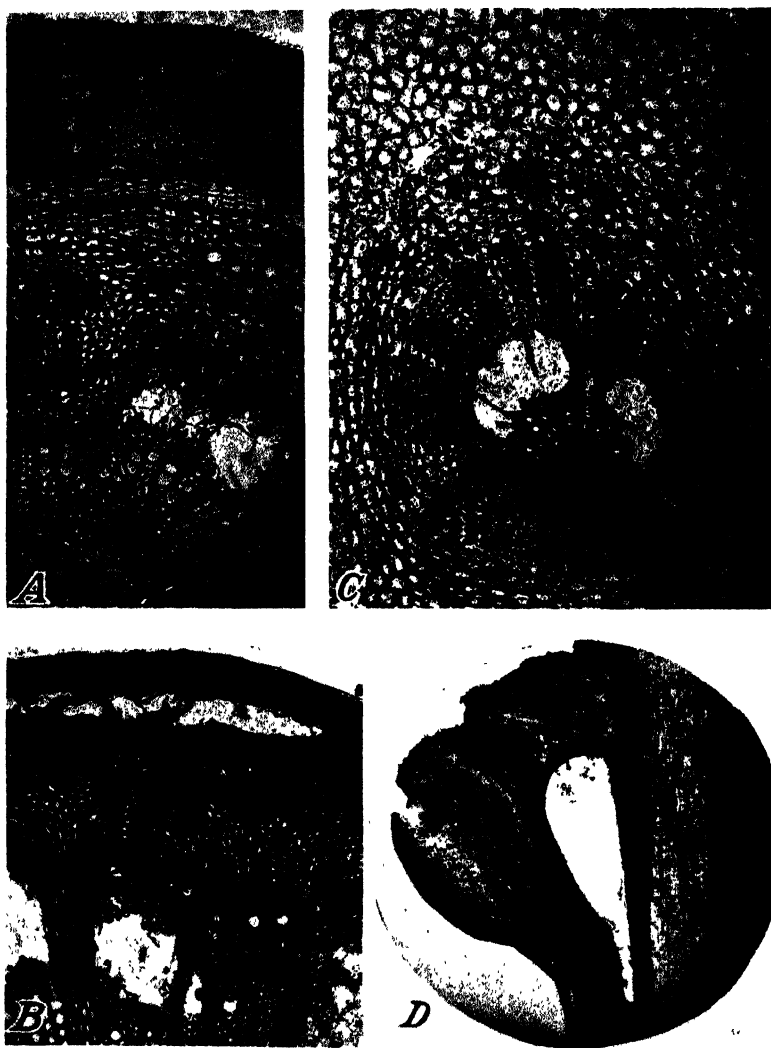
Cells of normal size and arrangement, showing various stages in the disorganization of their protoplasts, are visible in the discolored areas present at or near the margins of boron-injured lemon and grape leaves and in the larger veins and petioles of boron-affected grape, apricot, and prune leaves (pl. 1, *B, C, E, H*). In the early stages of this protoplasmic disintegration, the browned protoplasts completely fill the cell lumina, but later they often shrink away from the cell walls. Such browning of cells is at times observable in the epidermis, palisade parenchyma, spongy parenchyma, and parenchyma of the larger veins, but usually does not extend from upper epidermis to lower epidermis at a given point in the lamina. The conducting tissues of veinlets surrounded by necrotic mesophyll are frequently filled with brownish contents of gummy appearance.

Associated with such necrotic areas, very limited hypertrophied and hyperplastic areas are occasionally visible in the mesophyll of boron-injured lemon leaves (pl. 1, *F*) and in the petioles and midribs of boron-injured prune (pl. 1, *I*) and apricot leaves. At times a phellogen layer develops beneath the necrotic parenchyma of apricot and prune petioles (pl. 1, *K*) and midribs, resulting in the sloughing off of small, macroscopically visible scales of dead tissue. In prune petioles and midribs with externally visible injuries, gum pockets formed by disintegration of xylem elements may be present (pl. 1, *H*). The absence of changes in the vascular anatomy of boron-injured citrus leaves is reported by Haas (6).

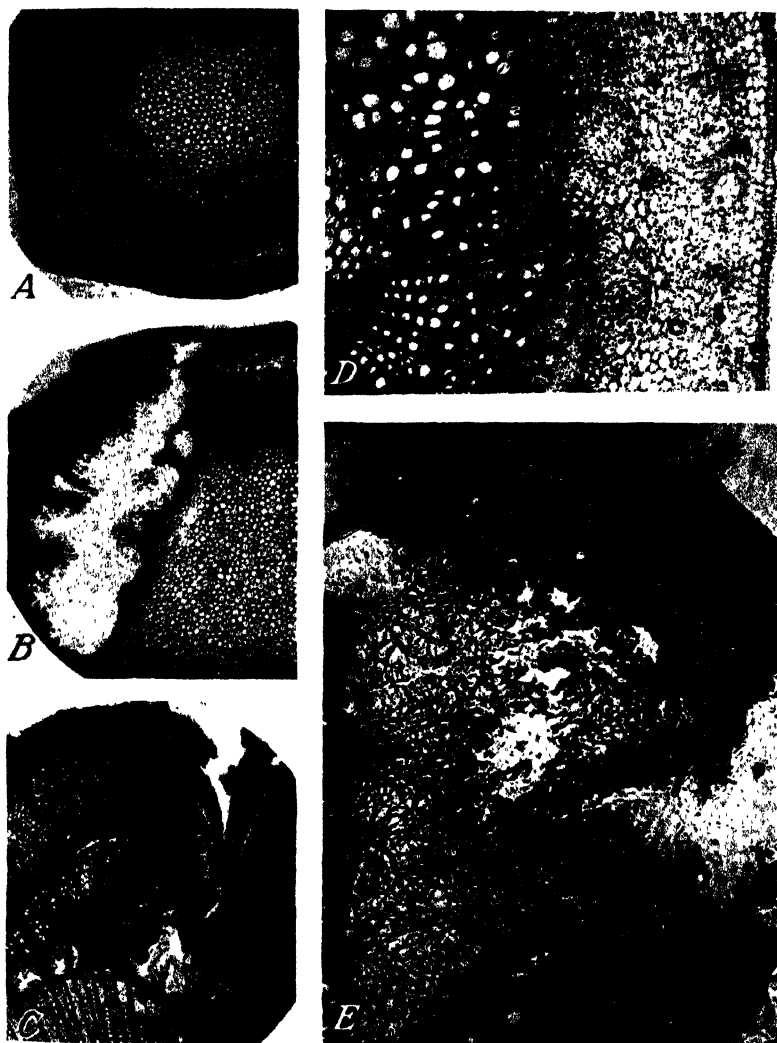
The only additional way in which boron-injured leaves were observed to differ histologically from comparable healthy leaves is in the presence of fewer calcium oxalate crystals in the injured leaves. This difference is most noticeable in the phloem of petioles and veins of apricot and prune leaves (pl. 1, *G, H*). In this connection it is noteworthy that Haas (6) reports that chemical analyses have shown that boron-injured lemon and walnut leaves contain a lower percentage of calcium and a higher percentage of potassium than healthy leaves of these species.



1-*F*, Cross sections of leaf blades: *A*, Healthy grape; *B-C*, boron-injured grape; *D*, healthy lemon; *E-F*, boron-injured lemon. $\times 50$ *G-I*, Cross sections of prune midribs: *G*, Healthy; *H I*, boron-injured. $\times 35$ *J-K*, Cross sections of prune petioles. *J*, Healthy, *K*, boron-injured. $\times 35$



A-B, Cross sections of internodes of current season's stems: *A*, Boron-injured prune; *B*, boron-injured peach. $\times 79$. *C*, Cross section of branch trace in node of boron-injured prune $\times 79$. *D*, Longitudinal section of node of boron-injured prune. $\times 9$



1-3, Cross sections of internodes of current season's stems of apricot. A, Healthy; B, boron-injured $\times 9$. C, Cross section of internode of 2-year-old stem of boron-injured prune. $\times 9$. D-E, Cross sections of internodes of current season's stems of peach. D, Healthy; E, boron-injured. $\times 79$.



A-B, Cross sections of nodes of current season's stems of apricot: *A*, Healthy, *B*, boron-injured. $\times 7.5$.
C, Longitudinal section of node of healthy apricot $\times 7.5$. *D-E*, Cross sections of nodes of current season's stems of prunes *D*, Boron-injured; *E*, healthy $\times 7.5$. *F*, Longitudinal section of node of boron-injured apricot $\times 7.5$.

ABNORMALITIES OF STEMS

The greatest abnormalities in stem structure of boron-injured stone-fruit trees are to be found in shoots that do not live beyond the first year, although enlarged nodes are often conspicuous on 2- and 3-year-old branches. In current season's stems of boron-affected prune, peach, and apricot trees, necrotic areas comparable to those discussed in connection with boron-injured leaves occur in the epidermis and subjacent cortical parenchyma (pl. 2, *A*). As in the case of petioles of these species, phellogen (pl. 2, *A*) may develop beneath the necrotic tissues which at times slough off in the form of small scales. Since the necrotic areas of cortical parenchyma are commonly lenticular in cross section and may extend deeply into the cortex, such phellogen formation is in contrast to that normally occurring in the subepidermal layer of cortical parenchyma cells. As in the leaves, the current season's stems of boron-injured stone-fruit trees, particularly those of peach and apricot, may contain noticeably fewer calcium oxalate crystals than comparable healthy specimens (pl. 3, *A, B, D, E*). This difference in calcium oxalate content is visible in cortex, phloem, and pith.

Small lysigenous cavities filled with gum are frequently present in the cortex of first-year stems of boron-injured stone-fruit trees (pl. 2, *B*; pl. 3, *B*). The pressure exerted by the gum in these cavities is often sufficient to rupture the epidermis, but at times merely distends the overlying tissues and causes minute swellings on the periphery of the stem. Although such cortical gum cavities are rather numerous, axial lysigenous gum ducts in the xylem are far more abundant and conspicuous (pl. 2; pl. 3, *B, C, E*). The gum ducts are apparently formed by the disintegration of abnormal xylem parenchyma. Such tissue at times persists and may form a large percentage of the wood normally composed of other axially elongated xylem elements (pl. 3, *E*). In the internodes the gum ducts in the xylem may encircle the stem. At the nodes, xylem elements of leaf and branch traces, as well as those of the main vascular cylinder, may disintegrate to form gum ducts (pl. 2, *C*). Occasionally cortical gum cavities and gum ducts in the xylem become united through the disintegration of intervening cambium, phloem, and cortical elements (pl. 3, *B, C*). Such fusion of gum cavities indicates that a large proportion of the gum which accumulates on the surface of these stems has its origin in the xylem. Gum pockets may also occur in the pith immediately beneath the dead portion of twigs which have died at the tips. Gum ducts similar to those occurring in first-year stems may also be formed in later years (pl. 3, *C*).

The enlargement of nodes of boron-injured stone-fruit trees (pl. 2, *D*; pl. 4, *B, D, F*) is chiefly due to the growth of axillary and accessory buds which would normally remain dormant (pl. 4, *C-F*). The branch traces of such nodes are therefore usually considerably larger than those in healthy stems. More or less hyperplasia commonly occurs in the cortex of the enlarged nodes (pl. 2, *D*; pl. 4, *A, B*), and hypertrophy of some of the parenchyma cells is not unusual. In the shortened internodes of boron-injured stone-fruit stems the cortical parenchyma cells remain shorter and exhibit less variation in size than similar cells in comparable healthy stems.

DISCUSSION

From the foregoing it is evident that the manifestations of boron toxicity which are characteristic of some plants are lacking in others. Butler (2) has cited numerous instances in which similar reactions of tissues may be brought about by widely different stimuli. Hence it is not surprising to find that the histological evidences of injury by boron are, in general, similar to those attributable to other causes. The enlarged nodes of boron-injured stone-fruit trees are not unlike those of French prune twigs affected with exanthema (14). Lysigenous gum ducts in stone-fruit trees have been induced by a wide variety of stimuli including a number of fungi, bacteria, insects, chemical substances, and traumatism (3). Priestley and Woffenden (12) have shown that phellogen formation in both normal and abnormal positions results from a set of conditions that may be fulfilled in many ways. The calcium oxalate content of the aboveground organs of plants is influenced by various factors which influence the rate of transpiration (10), as well as by the composition of the nutrient solution (1). Hypertrophied cells and hyperplastic areas in leaves may result from such diversified stimuli as fungi, insects, injurious vapors (2), and boron deficiency (7). Chloroplasts frequently change back into leucoplasts in starving cells and in those incited to abnormal growth (10). Localized browning of cells may be initiated by sunburn (4), wounding (10), or virus infection.

The cases cited above are sufficient to indicate that a toxic concentration of boron in the nutrient solution cannot be regarded as a stimulus to a specific reaction reflected as a specific histological change, but that the histological changes induced by excessive concentrations of boron are correlated with the inherent capacity of a species to respond to stimuli. Theoretically every living plant cell possesses the potential capacity to react to various stimuli by hypertrophy, hyperplasy, or the development of meristematic tissues (2, 10), but the potentiality is greater in certain tissues than in others, and these tissues show greater potentiality in certain species than in others. It has been suggested that one of the roles which boron plays in the plant is that of stimulating meristematic activity (1, 7, 8, 16, 17). Structural abnormalities resulting from stimulation to abnormal growth are met with in the stems, petioles, and veins of some boron-injured stone-fruit trees and to a very limited extent in boron-injured lemon leaves. Such of these structural abnormalities as occur in the cortex of stems, the parenchyma of petioles and veins, and the mesophyll of leaves are commonly associated with necrotic areas in which the cells are of normal size and arrangement. Similar necrotic areas are found in the mesophyll of boron-injured lemon and grape leaves which show no structural abnormalities. These necrotic areas are commonly preceded and bordered by areas in which partial degeneration of chloroplasts indicates a weakened condition of the protoplast.

Kelley and Brown (9) and subsequently Scofield and Wilcox (13) have shown that boron accumulates in the leaves of boron-injured citrus and walnuts. Eaton (5) has shown further that the tissues manifesting the greatest injury are those in which boron has accumulated in relatively high concentrations. It seems probable, therefore, that an excessive concentration of boron in the cell often injures the

protoplast to such an extent that it undergoes a progressive degeneration first affecting the chloroplasts. However, in some instances an excess of boron may stimulate the protoplast to abnormal growth or division.

SUMMARY

Injury to plants resulting from boron occurring in excessive concentrations as a natural constituent of irrigation waters is a factor of agricultural importance in certain areas in the western United States.

The macroscopic symptoms of boron injury to various plants have been described in previous papers.

Histological characteristics of boron-injured lemon, grape, prune, and apricot leaves, and prune, peach, and apricot stems are described herein.

In general, the plant parts manifesting the greatest injury are those in which boron has accumulated in relatively high concentrations.

Both macroscopic and microscopic manifestations of boron toxicity which are characteristic of some plants are lacking in others. This indicates that a toxic concentration of boron in the nutrient solution is not a stimulus to a specific reaction reflected as a specific histological change.

The histological evidences of injury by boron are, in general, similar to those attributable to other causes. This suggests that the observed abnormalities are correlated with the inherent capacity of a species to respond to stimuli.

It seems probable that an excessive concentration of boron in the cell often injures the protoplast to such an extent that it undergoes a progressive degeneration. Chloroplasts, when present, are affected first.

In some instances an excess of boron may stimulate the protoplast to abnormal growth or division.

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EFFECT OF VARIATIONS IN STAND ON YIELD AND QUALITY OF SUGAR BEETS GROWN UNDER IRRIGATION¹

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INTRODUCTION

In agronomic experiments, variations in stand between treatments or varieties are commonly encountered and must be taken into consideration at harvest. It is essential to know the growth response to such variations in order that the relative importance of any error resulting from them may be determined. This is true particularly for such crops as corn or sugar beets, which are either planted in hills or thinned to individual plants spaced at designated intervals in the row.

In this connection, certain published studies are of interest.

De Haan and Klijnhout (2)³ studied the relationship between density of stand and yield of sugar beets. They found a gradual decrease in weight per beet and a corresponding increase in total weight per unit area as the space allotment per plant decreased, the number of beets per acre varying from 16,000 to 45,000 while the distance between the rows was held constant at 40 cm. The studies were conducted on three fields of different degrees of fertility, the results being similar for all three.

Souček (6), in a study of the relation between stand and yield, in rows 42 cm apart, found a direct and positive correlation to exist within the observed limits of 11 to 38 beets per unit of row 8 m in length.

Willcox (7) reviewed the work of De Haan and Klijnhout (2) and Souček (6) and interpreted their results in relation to the Mitscherlich effect law of growth factors.

Munerati, Mezzadrolì, and Zapparoli (4) found no constant relation between the available space and weight per beet. Increased space available was found to be only partially compensated for by the increased weight of some of the beets.

Pack (5) obtained the correlation values 0.20 ± 0.03 for relative space with weight per beet and 0.21 ± 0.03 for space with sucrose content or total weight of sucrose per beet. He found no correlation, however, between space and percentage of sucrose.

Immer (8) studied individual variability of sugar beets grown without irrigation. He found weight of root to be much more variable than percentage of sucrose, and the regression of sucrose percentage on weight to be not entirely linear. The effect of variations in stand

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³ Reference is made by number (*italic*) to Literature Cited, p. 210.

were largely eliminated by removing before harvest the beets adjacent to noticeable skips.

It was the object of the studies reported in this paper to determine the extent to which the sugar beet will respond to additional space allotment per plant under irrigation in Colorado and to obtain a measure of the extent of the possible error which may be introduced into agronomic experiments as a result of variations in stand.

MATERIALS AND METHODS

Studies were conducted in 1930 on 2 irrigated beet fields near Fort Collins, Colo., and in 1931 on 2 near Fort Collins and 1 near Rocky Ford, Colo.

The results for 1930 are presented for the Barlow farm and the College farm near Fort Collins. The same commercial beet variety was used for both fields. With the exception of four comparisons made on the College farm, the rows were 20 inches apart, and the beets were spaced 12 inches in the row, the blocking having been done mechanically.

On the Barlow farm, all beets from a plot 22 rows wide by 132 feet long were harvested individually, and a record was made of the location of each beet with reference to blank spaces in the row and in the adjacent rows. Each beet was numbered and sampled with a rasp and analyzed for sucrose. The average weight and percentage of sucrose were computed for each lot having a particular type of competition, and comparisons were made with the check or "normally competitive" ⁴ beets on the basis of the standard-error values (experiment A in tables 1 and 2). A nearby portion of the same field was divided into 1,600 single-row plots 16 feet long (experiment A-1 to A-5 in table 6). The total number and the weight of beets were recorded for each plot, the data being used to determine the regression of weight of beets on stand. The type of competition was also recorded for every plant harvested. The relative response in beet weight to increased space allotment was determined from the portion of the same field harvested individually as described above. It was then possible to adjust the total weight of each plot having an imperfect stand to an expected value based on a perfect stand.

The regression of weight of beets on stand was also obtained for a uniformity study conducted in 1931 (experiment E, table 6), in which 1,280 plots were harvested in a manner similar to that described above for the 1,600 plots in 1930, except that no record was taken of the type of competition for individual beets.

On College farm in 1930 (experiment B in tables 1 and 3), the data were obtained entirely from paired comparisons. A "test" beet with a certain specific relation to one or more blank spaces in the same row or in adjacent rows was paired with the nearest check beet having competition on all four sides. So far as possible, the check beet always occupied the same relative position with reference to the test beet. The percentage sucrose determinations were made on bulked lots of beets representing each test-and-check comparison.

In 1931 there were 5 or 6 replications of each treatment at Fort Collins (experiment C in tables 1 and 4) and at Rocky Ford (experiment D in tables 1 and 5). Each "block" included several treat-

⁴ As used in this paper, "normally competitive" beets have competition from beets adjacent on 4 sides, while "noncompetitive" beets lack competition on 1 or more sides.

ments, the blocks being distributed at random in each of the replications.⁵ The Fort Collins field was planted by hand, with rows 20 inches apart and the beets spaced 12 inches apart in the row, the desired adjustments in stand being made at the time of thinning according to the plan shown in figure 1. At the Rocky Ford station the planting was done by machine; the width of row and spacing were the same as at Fort Collins, the accuracy of spacing being assured through the use of a marked tape.

The type of competition for the beets harvested in the various rows shown in figure 1 was as follows:

Row	Type of competition
A	Adjacent to one blank in adjacent row
A	Adjacent to two corner blanks in adjacent row
B	Between two blanks in same row
C	Between two blanks in adjacent rows
C	With four corner blanks
D	Check (singles surrounded by singles)
E	Blanks on four sides
F	Blanks on three sides
G	Adjacent to single blank in same row
H	Adjacent to single corner blank
I	Doubles surrounded by singles
I	Singles adjacent to doubles in same row and to singles in adjacent rows
J	Triples surrounded by doubles
J	Doubles adjacent to triples in same row and to doubles in adjacent rows

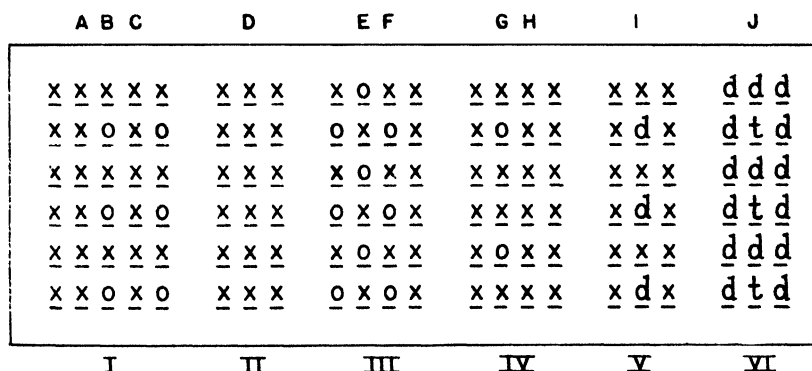


FIGURE 1.—Diagram of plan used for the competition study in 1931. I-VI, Blocks; A-J, rows, r, single plant; o, blank hill, d, double, i. e., 2 plants in one hill, t, triple, i. e., 3 plants in one hill.

The fertilizer experiment in 1931 (experiment F, table 6), data from which were used in calculating regression of weight of beets on stand, consisted of a randomized distribution of treatments and check plots. The original stand was not very satisfactory and the thinning labor was directed to leave as good a stand as possible. As a result there was a wide variation in spacing and uniformity of stand, although none of the differences between treatments, either for stand or tonnage of beets, was significant.

The width-of-row and spacing study (table 7) was conducted at Fort Collins in 1931. The seed was planted in hills with hand planters, the spacing being accurate. Competitive beets were harvested and tonnage yields were calculated on the basis of a perfect stand.

⁵ "Block" as used by Fisher (1) is synonymous with "replicate" as used here.

In most cases the beets were lifted with a mechanical lifter. Samples for analyses were washed, and each beet was rasped with a Keil rasp for a sample of pulp. The cold-water digestion method was used for percentage of sucrose in the beet. For apparent coefficient of purity determination one-half of each beet was ground by a multiple-saw grinder and a sample of juice obtained from the pulp in a hydraulic press. The percentage of sucrose in the juice was then determined by Horne's dry lead method and the apparent coefficient of purity calculated as the ratio of sucrose percentage to the corrected Brix.

The regression of weight of beets on stand was determined from the following formula:

$$W = \bar{w} + b(s - \bar{s}),$$

where W is the estimated weight of beets for any particular stand; \bar{w} and \bar{s} , the means for weight and stand, respectively; s , any observed stand; and b , the regression coefficient, calculated from

$$\frac{\text{Covariance } ws}{\text{Variance } s} \text{ or } \frac{S(ws - \bar{w}\bar{s})}{S(w - \bar{w})\bar{w}^2},$$

where S represents summation, for all experiments except A-1, A-4, and E-1, in which, with large numbers of plots, the data were distributed into frequency surfaces, the regression function b being calculated from

$$\frac{S(s - \bar{s})(w - \bar{w})}{S(w - \bar{w})^2}$$

with no removal of block effects.

Statistical evaluations of the significance of differences were obtained in all cases. The standard errors for the 1930 studies were calculated for each group by means of the formula

$$S.E. = \sqrt{\frac{(fd)^2}{n-1}}$$

where d =deviation from the mean, f =frequency, and n =number of individuals. Generalized standard errors were calculated for the competition studies (tables 4 and 5), and for the spacing study (table 7) by means of Fisher's "analysis of variance" (1). Unless otherwise stated, the 5-percent point was used as a minimum level of significance, the odds being at least 19 to 1 before significance is established.

The standard errors for mean weight per root for the 1930 results are given for each determination in tables 2 and 3. Since sucrose determinations were made only on bulked lots, standard-error calculations were not possible. The standard errors with the z test and difference necessary for significance for the 1931 results are given in tables 4 and 5.

EXPERIMENTAL RESULTS

ANALYSIS OF COMPETITION STUDIES

The results obtained are presented in tables 1 to 5. Table 1, which shows a general summary of results, includes only the number of beets taken and their weights in percentages of the check. For convenience in discussion, the four locations given in table 1 are designated A, B, C, and D.

TABLE 1.—Summary of results obtained from competition studies conducted at four locations in Colorado, 1930 and 1931

Type of competition	1930						1931					
	Barlow (A)			College (B)			College (C)			Rocky Ford (D)		
	Number of beets	Weight (percent-age of check)	Sucrose (percent-age of check)	Number of beets	Weight (percent-age of check)	Sucrose (percent-age of check)	Number of beets	Weight (percent-age of check)	Sucrose (percent-age of check)	Number of beets	Weight (percent-age of check)	Sucrose (percent-age of check)
1 corner blank	450	103 0	99 2	35	107 9	98 7	28	103 3	100 7	18	123 5	97 7
2 corner blanks	61	107 8	100 8	33	118 4	104 0	15	108 4	97 9	17	121 3	91 4
4 corner blanks	223	113 0	96 2	80	111 5	101 3	18	108 5	95 1	18	130 9	97 1
1 blank in adjacent row	203	126 8	99 2	97	120 8	102 0	31	105 4	96 3	18	130 9	97 1
1 blank in same row	11	134 8	100 8	41	141 3	96 4	24	138 6	95 8	18	120 3	96 6
2 blanks in adjacent rows	14	162 4	101 5	45	203 4	92 3	20	111 9	95 8	19	150 7	95 4
2 blanks in same row	17	201 9	96 2	33	180 2	97 4	23	182 0	95 8	22	178 7	94 8
Blanks on 3 sides	17	201 9	96 2	33	180 2	97 4	25	202 8	95 1	21	194 9	97 7
Blanks on 4 sides	17	201 9	96 2	33	180 2	97 4	22	105 1	97 9	18	129 6	100 0
Doubles ¹ surrounded by singles	17	201 9	96 2	33	180 2	97 4	17	79 5	101 4	15	94 7	98 9
Singles between doubles, same row	17	201 9	96 2	33	180 2	97 4	39	92 3	100 0	33	112 3	99 4
Average singles and doubles alternated	17	201 9	96 2	33	180 2	97 4	17	85 5	97 2	14	106 9	100 6
Doubles alternated with triples ²	17	201 9	96 2	33	180 2	97 4	16	75 1	96 6	16	124 8	102 9
Triangles surrounded by doubles	17	201 9	96 2	33	180 2	97 4	33	80 3	97 9	30	117 3	101 7
Average doubles and triples alternated	17	201 9	96 2	33	180 2	97 4	33	80 3	97 9	30	117 3	101 7
Check (singles alone)	1,189	100 0	100 0	1,189	100 0	100 0	25	100 0	100 0	36	100 0	100 0

¹ Average number per plot, 5 plots at College farm, 6 plots at Rocky Ford. Doubles and triples were counted as number of hills.

² Includes plants adjacent to 3 blanks on Barlow farm.

³ 2 beets per hill.

⁴ 3 beets per hill.

⁵ A different check with an average of 24 beets per plot was used for the comparison of doubles and triples alternated.

TABLE 2.—Summary of results obtained on the Barlow farm (location A), Fort Collins, Colo., 1930

Type of competition for beets harvested	Average number of beets	Weight of root		Percentage of sucrose	
		Grams	Percentage of check	Average percent	Percentage of check
1 corner blank.....	450	648± 16	103 0	12 9	99 2
2 corner blanks.....	61	720± 45	107 8	13 1	100 8
1 blank in adjacent row.....	233	755± 22	113 0	12 9	99 2
1 blank in adjacent row and 1 corner blank.....	90	841± 41	125 9	12 4	95 4
1 blank in same row.....	203	847± 24	126 8	12 9	99 2
1 blank in same row and 1 corner blank.....	75	800± 44	119 8	12 9	99 2
Between blanks in adjacent row.....	11	894± 162	133 8	13 1	100 8
Between blanks in same row.....	14	1,085± 108	162 4	13 2	101 5
1 blank in adjacent and one in same row.....	52	882± 42	132 0	13 1	100 8
1 blank in adjacent, 1 in same row, and 1 corner blank.....	17	861± 77	128 9	12 5	95 2
Adjacent to 3 or more blanks.....	7	1,349± 159	201 9	12 5	96 2
Check (all beets with competition on 4 sides).....	1,189	668± 10	100 0	13 0	100 0

TABLE 3.—Summary of results obtained at College farm (location B), Fort Collins, Colo., 1930

Type of competition for beets harvested ¹	Number of pairs	Root weight per beet			Percentage of sucrose		
		Test	Check	Percentage of check	Test	Check	Percentage of check
		Grams	Grams				
1 corner blank.....	35	572± 43	530± 50	107 9	15 6	15 8	98 7
2 corner blanks.....	33	662± 50	559± 46	118 4	15 5	14 9	104 0
1 blank in adjacent row.....	80	590± 30	529± 32	111 5	15 4	15 2	101 3
1 blank in same row.....	97	691± 33	572± 27	120 8	15 2	14 9	102 0
1 blank in same row (16 by 22 inches).....	122	750± 30	595± 25	126 1	14 3	14 9	96 0
Between blanks in same row.....	41	791± 62	560± 48	141 3	15 3	15 4	99 4
Between blanks in same row (16 by 18 inches).....	9	657± 117	515± 191	127 6	15 4	15 4	100 0
Between blanks in same row (16 by 20 inches).....	44	625± 94	516± 78	121 1	14 2	14 9	95 3
Between blanks in same row (16 by 22 inches).....	50	834± 54	676± 47	123 4	14 5	14 5	100 0
1 blank in same and 1 in adjacent row.....	72	812± 35	549± 34	147 9	15 1	15 6	96 8
Blanks on 3 sides, 2 in same, 1 in adjacent row.....	45	948± 60	466± 30	203 4	14 4	15 6	92 3
Blanks on 3 sides, 1 in same, 2 in adjacent rows.....	21	1,186± 70	644± 70	184 2	13 9	14 5	95 9
Blanks on 4 sides.....	33	838± 73	485± 41	172 8	14 7	15 1	97 4
Doubles surrounded by singles.....	88	736± 36	686± 32	107 3			
Triples surrounded by singles.....	10	757± 71	560± 59	135 2			

¹ Spacing and width of row 12 by 20 inches, unless otherwise specified

TABLE 4.—*Summary of results obtained at College farm (location C), Fort Collins, Colo., 1931*

[Values are given as means of 5 plots and percentage of check]

Type of competition for beets harvested	Average number of beets per plot	Weight of tops per beet		Weight of roots per beet		Percentage of sucrose		Apparent coefficient of purity	
		Grams	Percentage of check	Grams	Percentage of check	Average	Percentage of check	Average	Percentage of check
1 corner blank	28	530	104.7	826	103.3	14.5	100.7	90.2	100.7
2 corner blanks	18	520	102.8	875	109.4	14.1	97.9	89.6	100.0
4 corner blanks	22	502	117.0	876	109.5	13.7	95.1	88.0	98.2
1 blank in adjacent row	18	534	106.3	843	105.4	14.3	99.3	90.5	101.0
1 blank in same row	31	647	127.9	1,109	138.6	13.8	95.8	88.0	98.2
Between 2 blanks in adjacent rows	24	566	111.9	895	111.9	13.9	96.5	89.1	99.4
Between 2 blanks in same row	20	715	141.3	1,296	162.0	13.8	95.8	88.0	98.2
Blanks on 3 sides, 2 in same, 1 in adjacent row	23	877	173.3	1,453	181.6	13.3	92.4	88.2	98.4
Blanks on 4 sides	25	990	195.7	1,622	202.8	13.4	93.1	87.2	97.3
Doubles surrounded by singles	22	565	111.7	841	105.1	14.1	97.9	89.3	99.7
Singles between doubles, same row	17	423	83.6	636	79.5	14.6	101.4	89.5	99.9
Average singles and doubles alternated	30	494	97.6	738	92.3	14.4	100.0	89.4	99.8
Check, singles alone (A) ²	25	506	100.0	800	100.0	14.4	100.0	89.6	100.0
Doubles alternated with triples	17	508	112.4	625	85.5	13.9	97.2	89.8	100.6
Triples alternated with doubles	16	537	118.8	549	75.1	14.1	98.6	89.8	100.6
Average doubles and triples alternated	33	523	115.7	587	80.3	14.0	97.0	89.8	100.6
Check, singles alone (B) ⁴	24	452	100.0	731	100.0	14.3	100.0	89.3	100.0
2		1 4087		2 4099		0 4722		0 3208	
5-percent point		4096		4066		4066		4066	
1-percent point		5553		5553		5553		5553	
Difference for significance		88		110		73			
Standard error (percent of mean)		4.73		3.80		1.85		.82	

¹ Pairs² Applies to all treatments listed above in the table³ Hills⁴ Applies to the treatments included between checks A and B in the table.TABLE 5.—*Summary of results obtained at Rocky Ford, Colo. (location D), 1931*

[Values are given as means of 6 plots and percentage of check]

Type of competition for beets harvested	Average number of beets per plot	Weight of roots per beet		Percentage of sucrose		Apparent coefficient of purity	
		Grams	Percentage of check	Average	Percentage of check	Average	Percentage of check
2 corner blanks	18	463	123.5	17.0	97.7	86.4	98.3
4 corner blanks	17	455	121.3	15.9	91.4	86.1	98.0
1 blank in adjacent row	18	491	130.9	16.9	97.1	87.2	99.2
Between 2 blanks in adjacent rows	18	451	120.3	16.8	96.6	86.8	98.8
Between 2 blanks in same row	19	565	150.7	16.6	95.4	87.7	99.8
Blanks on 3 sides, 2 in same, 1 in adjacent row	22	670	178.7	16.5	94.8	86.6	98.5
Blanks on 4 sides	21	731	194.9	17.0	97.7	87.5	99.5
Doubles surrounded by singles	18	486	129.6	17.4	100.0	87.8	99.9
Singles between doubles, same row	15	355	94.7	17.2	98.9	88.2	100.3
Average singles and doubles alternated	33	421	112.3	17.3	99.4	88.0	100.1
Doubles alternated with triples	14	412	109.9	17.5	100.6	87.5	99.5
Triples surrounded by doubles	16	408	124.8	17.9	102.9	87.2	99.2
Average doubles and triples alternated	30	440	117.3	17.7	101.7	87.4	99.4
Check (singles alone)	36	375	100.0	17.4	100.0	87.9	100.0
2		1 4626		—0.4640		0 2534	
5-percent point		5265				5265	
1-percent point		7443					
Difference for significance		97					
Standard error (percent of mean)		7.36		1.99		.58	

It is possible, by applying the data presented in table 1, to determine the extent to which the beets adjacent to a single blank utilized the additional space. Since the essential data are not complete for location D, the results obtained for locations A, B, and C only are averaged. As an average of these three locations, a beet adjacent to 1 corner blank was increased 4.7 percent in weight, and a beet adjacent to 1 blank in the adjacent rows and 1 blank in the same row was increased in weight 10.0 and 28.7 percent, respectively. The eight beets surrounding a blank space were, therefore, so increased in weight that they compensated for 96.2 percent of the loss due to a single missing beet. It is obvious that several blank spaces together will result in proportionately less efficient utilization of the vacant area by the adjacent plants.

As an average of the A, B, and C locations, increases in weight of beet root of 28.7, 55.2, and 95.0 percent, respectively, were obtained for beets adjacent to 1 blank in the same row, between 2 blanks in the same row, and with blanks on 4 sides. The respective increases in area are 41, 82, and 100 percent. Corresponding slight decreases were obtained for percentage of sucrose, the average in percentage of the check being 99.0, 98.9, and 95.6 for the three groups. A beet adjacent to 2 blanks on the corner, 2 blanks in the adjacent rows, and 2 blanks in the same row showed increases in weight of 13.6, 22.0, and 58.4 percent, respectively, for an average of the A, C, and D locations. Corresponding sucrose values in percentage of the check, or 98.8, 98.0, and 97.6, were obtained. It is evident that while the weight of the beet was increased greatly there was only a slight negative response for percentage of sucrose and a possible similar response in apparent coefficient of purity with increasing space for development. The effect on the apparent coefficient of purity appears to be less than the effect on percentage of sucrose.

The effect of doubles was studied for the B, C, and D locations. In 1930, at the B location, doubles surrounded by singles were found to yield an increase in weight of marketable⁶ beets of 7.3 percent over single beets surrounded by singles, which will be termed the "check" beets. Corresponding increases of 5.1 and 29.6 percent were obtained for the C and D locations. The C location at Fort Collins and the D location at Rocky Ford were planned so that singles and doubles were alternated in the row, while adjacent rows were thinned entirely to singles. The singles alone, when alternated in the same row with the doubles, yielded only 79.5 and 94.7 percent of the yields of check beets for these respective locations. When the average for doubles and singles was considered, the corresponding yields in percentage of the check were 92.3 and 112.3, or an average of 102.3 for the two results. Neither of these deviations from the yield of the checks is as great as twice the standard error of a difference; consequently, they cannot be considered significant. Under the conditions of this test, the tonnage yield of marketable beets was not significantly affected when 25 percent of all hills were doubles.

When doubles were alternated with triples in the row, with the adjacent rows consisting of doubles, there was a significant loss in weight per hill of marketable beets at Fort Collins for both the doubles and triples as compared with the check beets. In relation

⁶ A beet $1\frac{1}{2}$ inches or more in diameter is considered marketable. By test it has been found that beets less than $1\frac{1}{2}$ inches in diameter have more than a 50-percent chance of being returned to the grower as "tare".

to the check, the corresponding weights of doubles and triples were 85.5 and 75.1 percent, respectively. At Rocky Ford, the respective weights for the doubles and triples were 109.9 and 124.8 percent of the checks. The increase in weight of triples over the check closely approached significance.

As a result of late planting and relatively unsatisfactory growing conditions at Rocky Ford the yield was unusually low as contrasted with somewhat better than average conditions at Fort Collins. The differences obtained for the two stations in relation to doubles and triples might well be expected under these conditions.

REGRESSION OF WEIGHT ON STAND

The regression of weight on stand was determined by means of data from the three sources, as discussed under materials and methods. The correlation and regression values are given in table 6.

On a perfect-stand basis, the respective yields on the three fields, A, E, and F, were 18.3, 20.4, and 19.2 tons per acre. They may be considered highly productive fields.

The correlation coefficients varied considerably, but were significant in every case as determined by Fisher's *t* test, with the 1-percent point as the minimum level of significance. It is apparent that there is a very material correlation between total weight of roots from a given area and the relative stand of beets. This should be of real concern to a commercial grower of sugar beets.

TABLE 6.—*Correlation and regression values for the relation between weight of beets and percentage of stand, Fort Collins, Colo., 1930 and 1931*

Year, experiment, and treatment ¹	Plots		<i>r</i>		Regression of weight on stand ²		Mean percentage stand	Range in percentage stand
	Number	Size	Within blocks	Total	Within blocks	Total		
1930								
A 1	1,600	1 row × 16 feet		+ 0 5389		1 31	85.3	25-113
A 2	800	1 row × 32 feet	+ 0 5533	+ 5485	1 39	1 36	85.3	47-103
A 3	200	4 rows × 32 feet	+ 3775	+ 3526	80	76	85.3	69-98
A 4	1,600	1 row × 16 feet		+ 6900		1 60	85.3	25-113
A 5	200	4 rows × 32 feet	+ 5361	+ 5824	1.24	1 33	85.3	69-98
1931								
E-1	1,280	1 row × 16 feet		+ 4386		1.08	89.0	31-100
E-2	160	4 rows × 32 feet	+ 3454	+ 1675	76	45	89.0	75-99
F ³	75	8 rows × 60 feet	+ 7098	+ 7375	2 10	1 37	59.5	33-78

¹ Experiments A and E were uniformity trials and F was a fertilizer study. Trials A-1, A-2, and A-3 were made before correction, and A-4 and A-5 after correction for competition. (See section on materials and methods.)

² The regression value is given as tons of beets for each 10-percent variation in stand.

³ The 4 central rows only were harvested for the F trial, in all other cases, the entire plot was harvested.

The regression of weight on stand has been converted into expected tons of beets for each respective 10-percent increase or decrease in stand. The total regression only was calculated for the original plots, 1 row 16 feet in length, for experiments A-1, A-4, and E-1. When two or more of the original plots were grouped into larger sizes of plots, then a certain arbitrary number of hypothetical treatments were assumed as follows: 20 treatments for A-2, and 5 treatments for A-3, A-5, and E-2, with 40 replicates in each case. There were 15 treat-

ments in the fertilizer trial, F, with 5 replicates of each treatment. Where treatments were assumed for the uniformity trials, A and E, or actually provided as in the fertilizer trial, it was then possible to assign certain portions of the variance to replicates and accordingly to reduce the total variance within replicates. The regression within blocks represents more nearly the true relationship since it is freed from the variation between blocks or replicates. In such cases, the regression was determined for both the total and within-blocks relationships. The regression within blocks was materially greater than the total regression for the E-2 and F experiments, indicating for these experiments considerable variation between blocks. There was apparently no appreciable block effect for the other studies.

The regression value is reduced when the plot size is materially increased for the uniformity trials, A and E, with a consequent increase of the range in percentage of stand. When the plot size for the A experiment was increased from 1 row 16 feet in length to 1 row 32 feet in length, the total regression value remained practically constant, being 1.31 and 1.36 tons, respectively, for each 10 percent in stand for the two plot sizes. An eightfold increase in size, however, for A-3 as compared to A-1, resulted in a decrease from 1.31 to 0.76 tons for each 10 percent in stand. Corresponding reductions in regression values from 1.69 to 1.33 tons were obtained for A-4 and A-5, and from 1.08 to 0.45 tons for E-1 and E-2, both of these constituting eightfold increases in size of plot.

It seems apparent on the basis of these results that each unit of increase or decrease in stand will result in a greater variation in yield when the stand is low than when a perfect stand is approached. Additional support for this conclusion is found in the graph of the straight regression lines (unbroken) for experiments A-1, E-1, and F (fig. 2). These lines were drawn for the percentage-stand arrays included in the test. By Fisher's *t* test, the deviations of the expected from the obtained means in each array were not significant for any of the three tests. The *z* values for the three tests (0.2003, 0.1770, and 0.2948) closely approached the expected 5-percent points for significance (0.2867, 0.2926, and 0.3520). With larger populations, particularly in the extreme arrays, it seems probable that significant deviations from a straight line could be shown.

It is evident (fig. 2) that the regression lines must pass through the zero points for percentage of stand and for tons of beets per acre. It is also evident that the yield of beets will not continue to increase indefinitely in direct proportion to the increase in percentage of stand. In fact, it appears for the width-of-row and spacing data, which are also presented in figure 2, that up to 8-inch spacing there is generally some increase in tonnage yields, with an indication of a slight reduction in yield from 8- to 6-inch spacing. The reduction in yield for 6-inch as compared to 8-inch spacing was not statistically significant in any case. It was definitely shown at Fort Collins (table 1), however, that when doubles and triples alternated in the row, with the border rows consisting of doubles, there was a significant loss in tonnage, and it may be concluded that the stand was too dense in this case for maximum tonnage. From these results, it would seem probable that with sufficient data covering the entire range of arrays i. e., from zero to 200- or 300-percent stand, it should be possible to show a gradual curve, with the weight of beets increasing directly

in proportion to the number of beets for the lower percentage stands. This rate of increase would become gradually less as competition became more of a factor until the maximum was obtained; then an actual decrease would result as the stand became more dense.

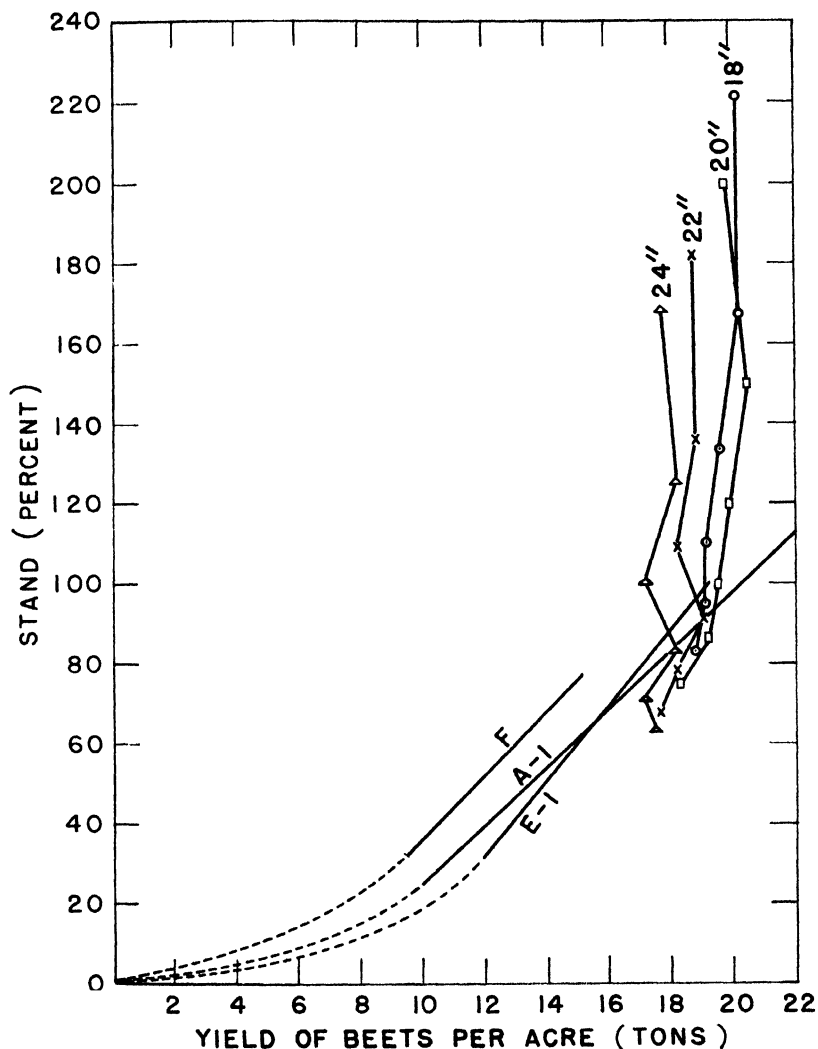


FIGURE 2.—Straight regression lines obtained for experiments A, E, and F (see table 6) with anticipated curvilinear projection (broken line) which intercepts the base lines for percentage of stand (26,136 beets per acre = 100-percent stand) and tons of beets per acre at the zero point. The mean yields for the width-of-row and spacing study, which are given in table 7 and plotted similarly in this figure, and the different values for each width of row are connected with straight lines. The yield is to a much greater extent dependent upon the density of the stand when the variation in stand is due largely to the presence of missing plants or skips (A-1, E-1, and F) than when the variation is due to uniform differences in spacing (18-, 20-, 22-, and 24-inch widths of row).

By applying the Mitscherlich effect law to the data of Souček (6) and De Haan and Klijnhout (2) Willcox (7) has shown that a gradual curve is to be expected up to a certain point of density of

stand, but that further increases in stand are not expected to alter the yield.

When correction was applied, on the basis of known competition, to the individual plot yields for the 1930 uniformity trial (A, table 6), the linearity of relationship was somewhat improved (fig. 3), as shown by the sums of squares for deviations of the expected from the obtained mean-array values. The obtained z values for deviations from a straight line are 0.2003 and -0.0401 for before and after correction, respectively, with a 5-percent point of 0.2867 for each. As shown by figure 3, the obtained mean much more closely approached the expected when correction had been made. While it is quite evident that the correction instituted materially improved the linearity of relationship between stand and yield, it is not feasible to consider such an arduous and costly method for practical plot work.

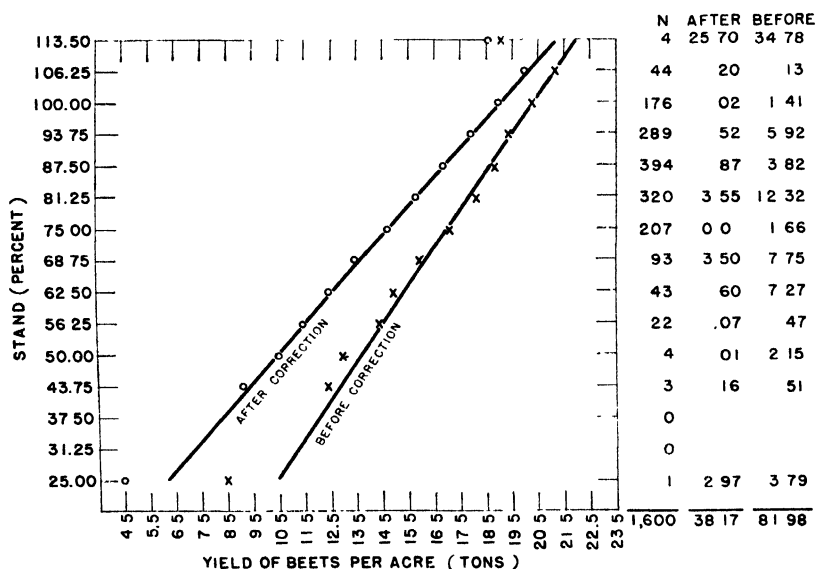


FIGURE 3.—Calculated straight regression lines for a uniformity trial at Fort Collins, Colo., 1930, with obtained mean yields in tons of beets per acre for each percentage-stand array. N, Number of observations; two right-hand columns show the sums of squares for deviations of the obtained from the expected for each percentage-stand array.

Results obtained in connection with a fertilizer experiment conducted on a farmer's field substantiate forcibly the relationship between the stand and tonnage yield. Strictly competitive beets, which were harvested from check or untreated plots in this particular experiment, averaged 13.54 tons per acre. The stand averaged 51 percent, and the field as a whole produced an average of 7.29 tons per acre, or 6.25 tons less than were obtained for the competitiveness. Assuming a regression of 1.25 tons of beets for each 10 percent in stand, the assumption being a fair one in relation to the results herein presented, a loss in tonnage of 6.13 tons per acre can be accounted for as due to a loss of 49 percent in stand. Figured at the local factory price paid to farmers for the 1932 crop, the farmer suffered a net loss of \$29.56 per acre from this loss in stand. The initial stand was excellent, the loss being almost wholly due to lack of diligence and care in

blocking and thinning and later cultivation. With careful work, the farmer should have harvested almost a perfect stand and nearly doubled the tonnage which he obtained, thereby realizing a net profit for his capital and labor invested.

RELATIONSHIP BETWEEN YIELD AND STAND

The mean yields for each of the treatments used in the spacing study are presented in table 7.

TABLE 7.—*Yield of beets per acre for competitive beets in spacing study, Fort Collins, Colo., 1931*

[Values are given as means of 6 plots]

Width of row (inches)	Yield when the spacing was -						Average yield
	6 inches	8 inches	10 inches	12 inches	14 inches	16 inches	
	Tons	Tons	Tons	Tons	Tons	Tons	Tons
18	20.10	20.28	19.60	19.13	18.02	18.77	19.48
20	19.86	20.50	19.96	19.58	19.17	18.31	19.56
22	18.73	18.79	18.17	19.07	18.25	17.67	18.45
24	17.71	18.26	17.21	18.17	17.13	17.51	17.67
Average	19.10	19.46	18.73	18.99	18.39	18.06	

The obtained z values for spacing, width of row, and interaction of spacing with width of row were 0.4769, 1.1082, and -0.5156 , respectively. The corresponding 5-percent points were 0.4176, 0.5950, and 0.4176, indicating significance for only spacing and width-of-row differences. It is obvious that the 18- and 20-inch widths of row produced a heavier tonnage of beets than the 22- and 24-inch widths when the stand was approximately the same. It is also apparent that, in general, the larger yields were obtained from the 8-inch spacing. The tonnage for the 6-inch spacing dropped off slightly, although not significantly, for each width of row. The increase in yield from the 8-inch spacing over that from the 12- and 16-inch spacing is shown in table 8.

There were 50 percent more beets per acre for the 8- than for the 12-inch spacing, and 100 percent more for the 8- than for the 16-inch. The corresponding average yield increases for the four widths of row were 0.47 and 1.39 tons of beets per acre, with a difference of 0.85 tons per acre necessary for significance. The 0.47-ton difference between the 8- and 12-inch spacing does not approach significance, while the 1.39-ton increase of the 8-inch over the 16-inch is highly significant. This provides a striking contrast to the regression within blocks, which ranges from 0.76 to 2.10 tons of beets per acre for each 10-percent increase in stand (table 6), the essential difference being that the variation in stand in the spacing study is due to uniform space allotment and also very largely to the presence of skips in the regression studies. The obvious conclusion is that uniformity of stand is a relatively far more important factor in determining final yield than is the particular width of row or spacing used. While it is not essential to this phase of the study, it is of interest to record that, although the tonnage of beets per acre is larger for the narrower spacings, the individual beets obtained were smaller and slightly higher in percentage of sucrose and purity.

TABLE 8. —Yield of beets per acre from 8-inch spacing as compared with yield from 12- and from 16-inch spacing, Fort Collins, Colo., 1931

Width of row (inches)	Increase or decrease (—) of yield per acre from 8-inch spacing as compared with that from —	
	12-inch spacing	16-inch spacing
	<i>Tons</i>	<i>Tons</i>
18	1.15	1.51
20	.92	2.19
22	.28	1.12
24	.09	.75
Average	47	1.39

DISCUSSION

The results reported indicate that the sugar beet is capable of marked response to variations in available space for development. Weight of beet is influenced to a much greater extent by an increase in the space allotment per plant than is either percentage of sucrose or apparent coefficient of purity.

In agronomic experiments with sugar beets the stand may be influenced by the type of treatment or differential response between varieties. Experiments which seek to determine the value of such factors as frequency and depth of cultivation, machine blocking as compared to hand blocking, time of planting, fertilizers or fertilizer-placement methods, irrigation, spacing studies, and thinning practices may be expected to show differences in stand between treatments with resulting variations in weight and quality of individual beets. In such cases the stand must be held constant in order to obtain a measure of the effect of the treatment. This may be obtained by harvesting only those beets which are surrounded by a full stand, i. e., normally competitive beets.

In actual practice it is clearly advantageous to have the beets either mechanically blocked or spaced by hand with reference to a marked tape. Metal boxing tape marked with lacquer is very useful for this purpose. It has been customary in the agronomic experiments to insist on competition on four sides. The data (table 1) indicate that although there is small response from 1 corner blank, 2 or more corner blanks may result in as large an error as 1 adjacent blank in the adjacent row. It is essential, therefore, to consider the presence or absence of beets on the corners in critical studies.

From the grower's standpoint it is of considerable interest to be able to present the yields on the acre basis regardless of the nature of the stand. This information may be obtained in addition to the perfect-stand data by harvesting the normally competitive beets (which are surrounded by perfect stand) separately from the noncompetitive beets (which lack competition in the same or adjacent rows). In practice the noncompetitive beets may be topped sufficiently with a hoe just previous to harvest to mark them. When lifted the competitive beets are first removed from the plot, leaving only the non-competitive beets to be taken up as a separate lot. The samples for

laboratory determinations are usually taken from the competitive beets in order to assure comparable results.

The regression of weight of beets on stand, if linear, would be useful in adjusting yields to a uniform-stand basis for treatment comparisons. From the regression values presented, it appears that this relationship was essentially linear within the observed stand limits. It is apparent, however, that this relationship would have been nonlinear with wider variations in stand (fig. 2).

The practical importance of skips in the stand was shown in the regression of weight of beets on stand. Three separate studies were made to demonstrate this relationship. Each 10-percent increase in the number of beets per unit area resulted in a corresponding increase of 0.76 to 2.10 tons of beets per acre (regression within blocks, table 6). This is in striking contrast with the results obtained from a spacing study (table 8), where there was an increase of 0.47 ton of beets per acre for a 50-percent increase in stand (8- over 12-inch) and 1.39 tons for a 100-percent increase (8- over 16-inch) when the spacing was consistent and uniform. These results demonstrate clearly the necessity of uniform stands for the commercial grower of sugar beets. The placement of major emphasis on uniformity of stand involves almost every field practice, particularly those operations preceding and immediately following blocking and thinning and, to a somewhat lesser extent, cultivation, irrigation, and protection against insect pests.

SUMMARY AND CONCLUSIONS

The studies here reported were conducted near Fort Collins, Colo., in 1930 and 1931, and at Rocky Ford, Colo., in 1931. The object was to determine the response of sugar beets to increased space allotment per plant, and the relationship which exists between weight and density and uniformity of stand.

On the basis of 2 years' results obtained at three locations, it was determined that the beets surrounding a single blank space were increased in weight to compensate for 96.2 percent of the loss of a single beet.

As an average of the three locations, increases in root weight of 28.7, 55.2, and 95.0 percent were obtained for beets adjacent to 1 blank space in the same row, between 2 blank spaces in the same row, and with blank spaces on 4 sides, respectively. The respective sucrose values in percentage of the check were 99, 98.9, and 95.6 percent, and increases in area 41, 82, and 100 percent.

It is evident from the data presented that beet weight was greatly influenced by the relative area available for development. As the size of the beet increased in response to increased spacing allotment, there was a slight negative response in percentage of sucrose and possibly in the apparent coefficient of purity, although the effect on the apparent coefficient of purity appears to be less than that shown for percentage of sucrose.

When doubles were alternated with singles in the same row and the adjacent rows consisted entirely of singles, the weight of the singles adjacent to doubles in the row appeared to be reduced slightly. The respective yields for the two locations of singles alternated with doubles were 79.5 and 94.7 percent of the check. The corresponding

percentage yields of 92.3 and 112.3 when both doubles and singles were included did not differ significantly from the check yield for singles.

At Fort Collins a significant decrease in tonnage resulted from alternating doubles and triples, the border rows consisting of doubles. The respective yields for the doubles and triples were 85.5 and 75.1 percent of the singles. At Rocky Ford the total yield for doubles and triples was greater than the check, although not significantly so.

Weight of beets was found to be significantly correlated with percentage of stand, the r values ranging from +0.3454 to +0.7098 for the relationship within blocks.

The regression of weight of beets on stand proved to be essentially linear for the three experiments, although in each case the deviations from regression were such that the obtained z values were only slightly less than the expected values for the 5-percent point. It is probable from the data presented that, with a wider range of stand, non-linearity could be demonstrated. For each increase of 10 percent in stand, there was a corresponding increase ranging from 0.76 to 2.10 tons of beets per acre for regression within blocks.

Yields obtained in connection with a study of width of row and spacing showed increases of an average of 0.47 ton for the 8- over the 12-inch spacing, or an increase of 50 percent in number of beets per acre, and 1.39 tons for 8- over 16-inch, or a 100-percent increase in number of beets per acre, the spacing being accurate and uniform. Uniformity of space allotment, or, in other words, the elimination of skips or blank spaces in the stand, appears to be relatively a far more important factor in determining final yields than the particular width between rows or the spacing between beets in the row.

Practical suggestions are made for harvesting experimental plots of sugar beets in order to eliminate the effect of variations in stand.

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STUDIES ON PROPERTIES OF THE CURLY TOP VIRUS ¹

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INTRODUCTION

The determination of properties of the curly top virus is somewhat more difficult than are similar determinations with many other viruses, as the percentage infection from artificial inoculation is too low to afford a critical test for the presence or absence of virus. The unsatisfactory results from artificial inoculations have forced investigators to rely on the natural insect vector, *Eutettix tenellus* (Baker), for the production of any considerable amount of infection.

Experimental work on the properties of the curly top virus was facilitated by the development of a method by Carter (5) ³ for artificially feeding the beet leaf hopper. This method consists in placing a liquid containing the virus in a bag made of animal membrane and allowing nonviruliferous leaf hoppers access to the outside of the bag. The leaf hoppers puncture this membrane and feed sufficiently on the liquid to acquire the virus. In this way the beet leaf hopper may be utilized to transfer virus from the liquid medium to susceptible tissue of beet plants.

Modifications of Carter's original method of feeding the beet leaf hopper have been devised and used with considerable success in studies on the properties of the virus by those interested in this field of research. Severin and Swezy (14) determined that the virus is filterable, and recently Severin and Freitag (13) published results of further investigations on properties of the virus. In the latter work it was shown that the virus may be recovered from expressed beet juice after a period of aging ranging from 8 to 100 days, the period apparently depending on the previous treatment of the juice and on the conditions under which the virus was preserved. Virus was recovered from beet juice diluted 1:2,000 and from macerated leaf hoppers diluted 1:22,000. The virus was inactivated by a 10-minute exposure at 80° C., but temperatures considerably below freezing apparently had no injurious effect. No virus was recovered from dried plant tissue nor from dried beet leaf hoppers.

Studies on the properties of the curly top virus were started at Riverside, Calif., the latter part of 1932 and were continued during the following 2 years. In this paper certain methods which have greatly facilitated the experimental work are described and the results of the investigation are presented.

METHOD OF MAKING TESTS FOR VIRUS

In all of the tests for the presence of active virus in this series of studies, beet leaf hoppers were used as the agents of transfer of virus from the liquid medium to seedling beets. Several types of cages for

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² The writer is indebted to J. M. Fife, biochemist, Division of Sugar Plant Investigations, Bureau of Plant Industry, for making the hydrogen-ion determinations reported in this paper.

³ Reference is made by number (italic) to Literature Cited, p. 240.

feeding leaf hoppers were tried, one of which, designed for feeding the insects with small quantities of exudate obtained from the beet plant, proved very satisfactory for use with all kinds of virus-containing media. Cages of this type were used for the most part and were constructed as follows:

Each feeding cage was made from an ordinary 4-ounce bottle having a diameter of approximately 2 inches. The bottle was cut into three parts and the top part discarded. The two remaining



FIGURE 1 - Cage used in artificial feeding of the beet leaf hopper.

parts were ground smooth on the cut surfaces. The middle portion, covered at one end with cloth and at the other end with Baudruche capping skin, served as a cage for confining the leaf hoppers. The bottom portion was used as a moist chamber over which the cage was set. Nonviruliferous beet leaf hoppers were placed in the cage through an opening in the cloth cover and subjected to a starvation period of 2 to 18 hours, depending on the temperature. At the expiration of the starvation period the cage was inverted and drops of the material to be tested for virus content were placed on the cappingskin. The cage was then quickly righted, so as not to disturb the drops of medium, and placed over a moist chamber (fig. 1). The cages were placed under lights in a hood at a temperature of 95° to 110° F. and the leaf hoppers allowed a feeding period of 4 to 6 hours. The leaf hoppers were then removed and, except as noted, caged singly on seedling

beets. The number of plants infected under these conditions was taken as a relative measure of the virus content of the material on which the leaf hoppers had fed.

SOURCES OF VIRUS

BET BEET JUICE

Juice expressed from sugar beet plants affected with curly top has been used as the source of virus in nearly all of the studies on properties reported up to the present time. This beet juice, when fed directly to the beet leaf hopper, is not an especially favorable liquid from which to obtain virus, since only a small percentage of non-viruliferous beet leaf hoppers become viruliferous as a result of such feeding. Some estimate of the proportion of leaf hoppers that may be expected to acquire virus from beet juice may be obtained from

results selected from one of the experiments of Severin and Freitag (13), which seems to be representative. Using approximately 33 artificially fed leaf hoppers for inoculating each plant, they obtained 4.6 percent infection from leaf juice, 46.6 percent from root juice, 11.5 percent from leaf juice diluted with 5-percent sugar solution, and 4.3 and 42.1 percent, respectively, from leaf and root juice centrifugalized at high speed. These results indicate that even with the higher percentages of infection the actual number of the artificially fed leaf hoppers which picked up virus was small, since it is well known that a single viruliferous leaf hopper is able to transmit disease to a considerable percentage of the plants on which it feeds.

The limited capability of the leaf hopper for picking up virus from beet juice is further illustrated by the results shown in table 1, in which less than 4 percent of the total number of leaf hoppers given access to leaf and root juice and caged singly on seedling beets transmitted disease.

TABLE 1.—Relative availability to beet leaf hoppers of virus in leaf juice, root juice, and phloem exudate of sugar beets affected with curly top

Beet no.	Leaf juice		Root juice		Phloem exudate	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	Number	Number	Number	Number	Number
1	80	3	80	5	80	26
2	20	0	20	0	20	5
3	20	0	20	0	20	7
4	20	0	20	1	20	14
5	20	1	20	0	20	14
Total	160	4	140	6	160	66

Direct utilization of the expressed juice of diseased plants, because of the limited feeding which takes place, the high mortality of the insects, and other circumstances, involves the use of excessively large numbers of beet leaf hoppers to secure results, and the results are often contradictory and difficult of interpretation. Nevertheless, the method may be used successfully in the study of certain properties of the curly top virus. It is obviously unsuited for studies in which chemical agents on which the leaf hopper will not feed or which are injurious to the leaf hopper are added to the liquid containing the virus. In any type of property studies the method involves the exercise of a great amount of patience and effort and necessitates the use of enormous numbers of leaf hoppers.

Because of these obvious shortcomings of beet juice as a source from which beet leaf hoppers may acquire virus, the first efforts in this series of property studies were directed toward securing liquids of high virus content more acceptable to the beet leaf hopper than beet juice has proved to be. Attempts were made (1) to obtain the liquid content of the phloem of the beet and other plants in which there is reason to believe virus occurs in relatively high concentrations and (2) to separate the virus in expressed beet juice from materials that are toxic or unacceptable to the beet leaf hopper.

PHLOEM EXUDATE

According to the available evidence (2) the curly top virus occurs in the phloem of diseased plants in much greater concentrations than in other tissues. Since it has been shown also that the beet leaf hopper seeks the phloem elements in its feeding, the phloem content of diseased plants should constitute the best possible medium from which to recover virus when the beet leaf hopper is used as the agent of transmission.

Many varieties of cucurbits are susceptible to the curly top disease. Since these plants are known to give off considerable quantities of exudate from the phloem when the stems or petioles are cut, preliminary tests of phloem exudate as a virus-carrying medium were

made with Hubbard squash (*Cucurbita maxima* Duchesne) as the source of exudate. However, it was found that the exudate from this and other types of cucurbits coagulates quickly upon exposure to air and even when considerably diluted with water or 5-percent sugar solution it is not a favorable food for the beet leaf hopper. After having access to the phloem exudate from squash for 4 hours the leaf hoppers were weak and emaciated. However, of the 120 leaf hoppers exposed for 4 hours to this material and then caged singly on healthy seedling beets, 15 produced infection.

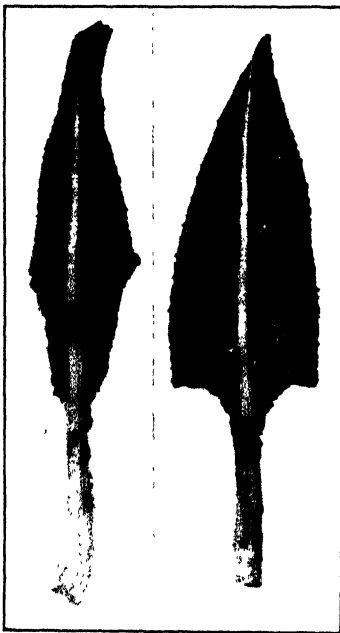


FIGURE 2.—Exudate on diseased beet leaves.

Attempts were made next to obtain exudate from the phloem tissue of sugar beets, *Beta vulgaris* L. Under certain conditions of rapid growth and high humidity, drops of clear or colored liquid appear on the blades and petioles of diseased beets (fig. 2). A small quantity of this exudate was collected by means of capillary tubes and tested by allowing leaf hoppers to feed on it and then transferring them singly to seedling beets. This exudate proved to have a high virus content and, if diluted sufficiently with water, served as a very satisfactory food for the beet leaf hopper. Exudate can be obtained only in very limited quantities from diseased blades and petioles. However, it was found that by properly regulating environmental conditions exudate could be obtained in larger quantities from the cut surface of both diseased and healthy beet roots.

After considerable testing of conditions which favor exudation the following method was adopted as best suited to this purpose. Beets 2 to 6 inches in diameter were removed from the soil, washed, topped, placed in sand in a container, and covered to avoid excessive evaporation. The tops of the beets projected 2 to 3 inches above the surface of the sand. The sand was kept wet but sufficient drainage was pro-

vided to prevent water-logging. A thin layer of tissue was removed from the top of each beet. On the cut surface drops of exudate appeared above the veins as shown in figure 3. These drops were collected by means of small glass tubes. Beets, if freshly cut, will yield exudate each day for as long as 3 weeks. The largest yield was always obtained after the first cut, and the volume of flow decreased progressively as other cuts were made. As a rule it is not desirable to cut beets more than once or twice each day. There is no evidence that the virus content of the exudate is influenced by the number of times the beet is cut or by the time it stays in sand so long as it remains sound. Tests showed that the leafhoppers fed readily on this exudate and that a large percentage acquired virus, as was proved by their ability to cause infection. Table 1 shows comparative results in acquiring virus by lots of leafhoppers allowed to feed on exudate from sugar beets affected with curly top and on expressed juice from leaves and roots of the beets from which the exudate was derived.

The available evidence as to the origin of the exudate obtained as described above indicates that it is derived chiefly from the phloem. Crafts (7) and others

have shown that a positive pressure normally exists in the phloem of certain plants and that liquid content escapes when phloem is tapped. The appearance of drops of exudate on the petioles and blades of diseased beets suggests that phloem content is extruded to the exterior as a result of pressure. Esau (8) presented results of histological studies which show that liquid moves to the surface



FIGURE 3.—Drops of exudate on cut surface of diseased beet.

from the region of phloem through the intercellular spaces, indicating strongly that the natural exudate on blades and petioles is derived from the phloem.

The exudate from the cut surface of beets collects in beadlike drops at the severed ends of the vascular bundles, indicating clearly a vascular origin. Evidence has been presented (2) indicating the non-occurrence of virus in the xylem elements of the vascular bundle. The high virus content of the exudate from sugar beets affected with curly top, therefore, is evidence that it is not derived from the water-conducting tissue but from the phloem. Moreover, exudation is not of the type which commonly results from injured xylem. Exudation from the cut surface of beets continues for a period of 30 minutes to 2 hours following cutting. Further exudation can be induced only by making fresh cuts. This suggests a closing of the outlets by coagulable materials of the phloem, a phenomenon that occurs to a more marked degree in cucurbits where, after injury, exudation continues for only a few minutes but can be induced repeatedly by making fresh cuts. Bleeding from the xylem would be expected to continue over a longer period.

The exudate from the cut surface of diseased beets closely resembles the natural exudate from the petioles in viscosity, hydrogen-ion concentration, oxidation reactions as indicated by color changes, total solids, and virus content. It seems logical to assume that naturally occurring exudate is derived from the phloem and that the exudate from the cut surface of beets is made up of the content of phloem tissues except for a certain amount of unavoidable contamination from injured cells of other tissues mainly parenchymatous in nature. The high virus content of phloem exudate of curly top diseased beets and its acceptability to the beet leaf hopper make this material an extremely satisfactory source of curly top virus. It has been used extensively in all experiments in which only small quantities of virus-containing liquid were required. In experiments requiring a considerable volume of liquid other materials have been used.

PRECIPITATES FROM BEET JUICE

Separation and purification studies by a number of investigators have shown that viruses have an affinity for certain types of precipitates formed upon the addition of certain chemical compounds to plant juices. Allard (1) found that the virus of tobacco mosaic is contained in the precipitate formed from tobacco juice when acetone or alcohol is added. Vinson (16) developed a method for purifying the virus of tobacco mosaic from the precipitate formed when safranine is added to the expressed juice of tobacco.

The addition of alcohol or acetone to beet juice results in the production of a precipitate that is easily removed by centrifugalization. It was found that this precipitate contained considerable virus that was released in a supernatant liquid after the precipitate was dried to remove the alcohol, suspended in water, and centrifugalized. Making use of this principle, the following method was developed for preparing virus-containing liquids for leaf hopper feeding. Juice from diseased beets is added to an equal volume of 95-percent alcohol. The resulting precipitate is thrown down by centrifugalization and the supernatant liquid, which contains most of the materials of the juice that

are unacceptable to the beet leaf hopper, is discarded. The precipitate is washed in 50-percent alcohol, dried, suspended in 5-percent sugar solution, and centrifugalized. The supernatant liquid from this second centrifugalization contains considerable virus and is very acceptable to the beet leaf hopper. The percentage of leaf hoppers that acquired virus from such a medium varied with the different preparations but was sufficiently high to justify the use of the medium in experiments in which phloem exudate could not be used because of the relatively large quantities of liquid required.

It was found that this method of separating the virus from materials that interfere with leaf hopper feeding could be used also with phloem exudate, ground beet leaf hoppers suspended in water, and with other virus-containing liquids which have proteins or other compounds that are precipitated in an alcoholic solution. The method has been of considerable value in separating the virus from toxic materials of the beet juice and from liquids to which various chemical compounds were added to determine their effect on the virus.

PRECIPITATION AND PURIFICATION

A number of tests were made to determine the completeness of the removal of the virus by the precipitate formed in beet juice and phloem exudate upon the addition of alcohol or acetone, and also to determine insofar as possible the completeness of release of the virus when the precipitate is thoroughly shaken in water or in 5-percent sugar solution. For these tests juice was pressed from badly diseased beet tops that previously had been frozen. A volume of 95-percent alcohol equal to the volume of juice was added and the resulting precipitate thrown down by centrifugalization. The precipitate was washed once with a volume of 50-percent alcohol equal to twice the volume of juice. It was then dried and made up to the original volume of juice with 5-percent sugar solution. This was shaken well and centrifugalized, and the supernatant liquid was reserved for testing. The precipitate was washed with 5-percent sugar solution a second and a third time. Tests were made to compare, as a virus source, (1) the original beet juice, (2) supernatant liquid after alcoholic precipitation, (3) supernatant liquid from the 50-percent alcoholic wash, (4) supernatant liquid from the first 5-percent sugar solution wash, (5) supernatant liquid from the second 5-percent sugar solution wash, and (6) supernatant liquid from the third 5-percent sugar solution wash. The alcohol was removed by evaporation from the supernatant liquid and the precipitate in (2) and (3).

Table 2 shows the results of a series of tests in which nonviruliferous leaf hoppers were fed on these preparations. As in previous experiments, the leaf hoppers were able to obtain very little virus from the untreated beet juice. No virus was obtained from the supernatant liquid following the alcoholic precipitation and little was recovered by washing the precipitate with 50-percent alcohol. These two liquids were evaporated to dryness and the residues made up to the original volume of beet juice with 5-percent sugar solution. They were not particularly distasteful to the leaf hoppers, although they were not so acceptable, apparently, as the other preparation. The fact that under these conditions the leaf hoppers picked up very little virus indicates that the removal of virus from the leaf juice by alco-

holic precipitation is practically complete and also that the precipitate may be washed in 50-percent alcohol without appreciable loss of virus.

A considerable quantity of virus is released from the alcoholic precipitate when it is washed with a 5-percent sugar solution, but a considerable quantity also is retained as shown in the tests of the second and third 5-percent sugar solution washes. Tests in which the second and third washes were used as virus sources gave almost as much infection as was obtained by using the first wash.

Experiments similar to the foregoing were made with phloem exudate as the original source of virus. As in the foregoing experiment, the removal of the virus by precipitation with alcohol was almost complete. The precipitate was washed six times with 5-percent sugar solution. Tests indicated that there was almost as much virus in the sixth wash as in the first.

Less extensive experiments indicate that acetone is about as effective as alcohol in precipitating the virus. Virus is released from the acetone precipitate, as from the alcoholic precipitate, upon the addition of water or 5-percent sugar solution.

TABLE 2.—*Leaf juice compared with alcoholic precipitate of leaf juice subjected to different treatments, as a source of virus for beet leaf hoppers*

[20 plants were used in each test with each type of inoculum]

Test no.	Plants infected from indicated virus source ¹				
	Leaf juice	Alcoholic wash of precipitate ²	First wash of precipitate ³	Second wash of precipitate ³	Third wash of precipitate ³
	Number	Number	Number	Number	Number
1	0	0	5	1	0
2	0	0	9	5	1
3	0	0	11	17	10
4	0	1	13	11	11
5	0	1	11	7	3
6	1	0	11	16	14

¹ No infections resulted when supernatant liquid from alcoholic precipitate was used as source of virus.

² Alcohol was removed by evaporation.

³ 5-percent sucrose solution was used for washing.

When alcoholic precipitates of beet juice or phloem exudate are suspended in 5-percent sugar solution and centrifugalized, the supernatant liquids usually retain enough material in suspension to produce a slight cloudiness. Some material remained in suspension even after prolonged centrifugalization. However, visible suspensoids were removed by passage of the supernatant liquids through Berkefeld and Mandler candles, and since the filtrates contained active virus, these visible suspensoids are apparently not essentially associated with the virus, although they may have some correlation with virus concentration in these preparations.

Virus-separation tests were made also by the safranin precipitation method developed by Vinson (16) for purifying tobacco mosaic virus. Juice expressed from previously frozen leaves of diseased beets was centrifugalized to remove the coarser particles. Five cubic centimeters of a 1-percent aqueous solution of safranin was added to 25 cc of expressed juice. The resulting precipitate was thrown down in a centrifuge, washed with a volume of water equal to that of the juice, and

suspended in 25 cc of distilled water. One gram of Lloyd's alkaloidal reagent was added, and the mixture was shaken at intervals for 30 minutes. It was then centrifugalized and the supernatant liquid, if almost clear, was poured off and tested for virus content. If safranin was still present in too great a quantity, the treatment with Lloyd's reagent was repeated. In most instances it was possible to obtain a straw-colored or slightly pink liquid. One gram of sugar was added to 25 cc of supernatant liquid, and tests were made for virus content by means of nonviruliferous leaf hoppers. Other tests were made in which phloem exudate was used instead of beet leaf juice.

TABLE 3. *Virus available to beet leaf hoppers in supernatant liquids from different precipitates*

Source of supernatant liquid for virus tests	Plants inoculated	Plants infected
	Number	Number
Safranin precipitate.....	60	1
Alcohol precipitate.....	60	28
Acetone precipitate.....	60	20
Alcohol precipitate and Lloyd's reagent..	20	14

The results (table 3) indicate that the leaf hoppers were able to acquire very little virus from the supernatant liquid from the safranin precipitate. As controls on the technic, supernatant liquids from alcohol and acetone precipitates were used and it was found that the leaf hoppers acquired considerable virus. The reason for the failure to obtain more virus from the supernatant liquid of the safranin precipitate is not clear. The leaf hoppers fed reasonably well on this material, though not so satisfactorily as on the supernatant liquids from the alcohol and acetone precipitates. This poorer feeding on the part of the leaf hoppers was apparently due to something released from Lloyd's reagent, since 1 g of Lloyd's reagent shaken in 25 cc of supernatant liquid prepared from an alcohol precipitate showed lower infections than an alcohol precipitate treated similarly except for the use of Lloyd's reagent. However, from this latter liquid leaf hoppers acquired considerable virus and it seems unlikely that failure to acquire more virus from the supernatant liquid following the safranin precipitate was due entirely to a lesser volume of liquid that may have been taken up by the leaf hoppers. It seems probable either that the virus is not carried down in the safranin precipitate or that it is not released from the precipitate upon the addition of Lloyd's reagent. Because of the difficulties inherent in the use of leaf hoppers as agents in transmission, no tests adequate to solve this problem have been devised.

Other tests show that the precipitates formed as a result of the addition of such reagents as calcium chloride and hydrochloric acid and the precipitate formed by heating juice or exudate to 60° C. carry down the virus. As judged from the percentage of infection, however, the amount of virus obtained from such precipitates has been apparently much less than from precipitates formed by the addition of alcohol or acetone.

DILUTION TESTS

A study of the infectivity of different dilutions of extracts of diseased plants and of ground viruliferous beet leaf hoppers was undertaken primarily to determine the dilutions of virus-carrying media that could best be used in further experimental work. In view of the fact that phloem exudate, the material that had proved to be the best source of virus, is available only in limited quantities, it was especially important to know how much this material could be diluted without seriously decreasing virus availability to leaf hoppers. The following materials and procedures were used in preparing liquids of different virus dilutions for leaf hopper feeding.

(1) Phloem exudate was collected from the cut surface of badly diseased beets and dilutions were made with 5-percent sugar solution.

(2) Beet juice was expressed from badly diseased beets selected from greenhouse material. A known volume of juice was added to an equal volume of 95-percent alcohol. The resulting precipitate was thrown down by centrifugalization, washed once with 50-percent alcohol, dried, and made up to the original volume of juice with 5-percent sugar solution. The precipitate was suspended in the sugar solution and dilutions were made direct from this mixture.

(3) One-tenth of a gram of viruliferous beet leaf hoppers was ground in a mortar and mixed with 2 cc of distilled water. The mixture was centrifugalized and the supernatant liquid was added to 2 cc of 95-percent alcohol. The resulting precipitate was thrown down by centrifugalization, washed once with 50-percent alcohol, dried, and suspended in 1 cc of 5-percent sugar solution. This was considered a 1:10 dilution and was used as the basis for higher dilutions.

Nonviruliferous leaf hoppers were allowed to feed on different dilutions of these preparations of phloem exudate, beet juice, and beet leaf hoppers, and, except in the case of high dilution, as noted in table 4, they were caged singly on seedling beets. The following numbers of plants were inoculated from each dilution, using one leaf hopper per plant: 184 from phloem exudate, 160 from beet juice, and 120 from beet leaf hoppers. The results of the dilution tests with phloem exudate are shown in table 4. The infection percentages obtained from dilutions of phloem exudate, beet juice, and leaf hoppers are shown in figure 4.

TABLE 4.—*Effect of dilution of phloem exudate on the number of artificially fed leaf hoppers that acquired and transmitted virus*

Magnitude of dilution	Plants infected by—		Magnitude of dilution	Plants infected by—		Magnitude of dilution	Plants infected by	
	1 leaf hopper per plant ¹	10 leaf hoppers per plant ²		1 leaf hopper per plant ¹	10 leaf hoppers per plant ²		1 leaf hopper per plant ¹	10 leaf hoppers per plant ²
None	Number	Number		Number	Number		Number	Number
1:5	57	-----	1:50	53	-----	1:10,000	0	4
1:10	72	-----	1:100	37	-----	1:20,000	-----	1
1:20	76	-----	1:500	18	-----	1:50,000	-----	0
1:20	60	-----	1:1,000	9	13			

¹ 184 plants were inoculated in each test.

² 40 plants were inoculated in each test.

Assuming that the percentage infection from different dilutions of phloem exudate, beet juice, and beet leaf hoppers, shown in figure 4, roughly represents comparative virus concentration, it is evident that the virus content of phloem exudate is much greater than that of beet juice and somewhat greater than that of the beet leaf hopper.

The maximum dilutions of beet juice from which virus was obtained correspond closely to those from which Severin and Freitag (13) consistently obtained virus, though the maximum dilutions from which they occasionally obtained virus are somewhat higher than the maximum reported here. These investigators showed that the maximum dilutions of beet juice from which virus was obtained by feeding the leaf hoppers directly on the beet juice varied with the method of preparation and treatment of the juice before leaf hopper feeding. In three types of juice treatment the maximum dilution from which they obtained virus was 1:100 from untreated juice as expressed

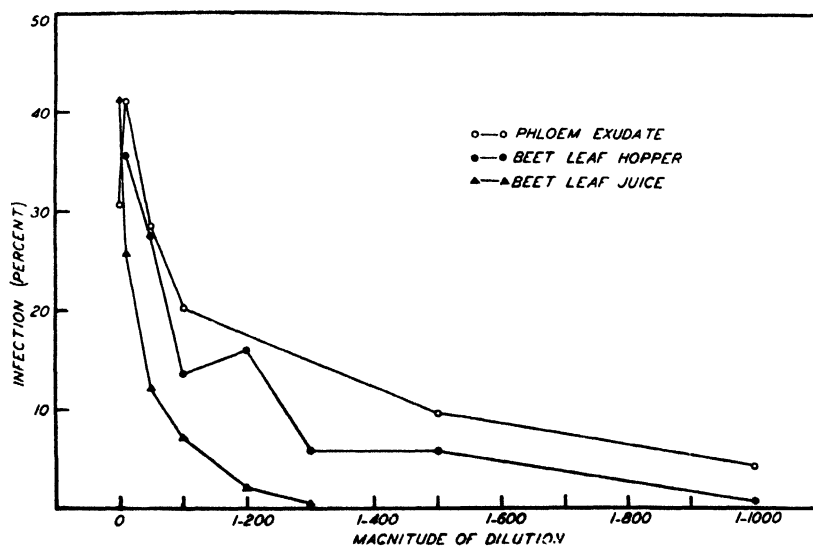


FIGURE 4.—Influence of dilution of virus from different sources on curly top infection.

from the plant, 1:1,000 from centrifugalized juice, and 1:300 from juice passed through a Berkefeld V or W filter. In each case a slight dilution was more favorable for virus recovery than was undiluted juice.

In comparing the results obtained in these tests it should be noted that Severin and Freitag (13) made their inoculations by caging approximately 33 artificially fed leaf hoppers on each plant. In the writer's tests only one insect was caged on each plant. The number of insects used to inoculate the test plant apparently influences infection and determines to some extent the maximum dilution from which virus may be recovered. This is illustrated by the results in table 4, which show that with phloem exudate as the virus source and by using 1 leaf hopper for inoculating each plant, virus was obtained from a dilution of 1:1,000 but not from a dilution of 1:10,000. By using 10 leaf hoppers for inoculating each plant, virus was obtained from dilutions of 1:10,000 and 1:20,000 but not from a dilution of 1:50,000.

These results indicate that mass action may be a factor influencing infection. Evidence of the operation of this phenomenon in infection by the curly top virus was first presented by Carsner and Lackey (4), who postulated a minimum infective dosage of virus. If it were assumed that some or all of the leaf hoppers that feed on a 1:10,000 dilution of phloem exudate acquired a small amount of virus but that the amount acquired in each instance was below the minimum required for infection, it follows that no infection would result from leaf hoppers caged singly, assuming no increase of virus in the insect. However, if 10 of these leaf hoppers were caged on a single plant the sum of the virus aliquots introduced by the different leaf hoppers might reach the minimum dosage necessary for infection. Although the results presented here are much too meager to justify conclusions, the method of approach seems to hold possibilities for an attack on some of the phases of the mass-action problem.

FILTRATION TESTS

Severin and Swezy (14) demonstrated that the virus in the juice of beets affected with curly top and that present in an aqueous suspension from macerated viruliferous beet leaf hoppers readily pass the common types of "bacteria-proof" filters. Since this work proves the filterable character of the curly top virus, it has not been thought worth while to make further extensive filtration tests. Such experiments as are recorded here were made preliminary to tests of longevity of the virus in filtered and unfiltered media.

For this preliminary work phloem exudate was diluted 1 part exudate to 9 parts 5-percent sugar solution and passed through Berkefeld N and W and Mandler medium and fine filters, under a pressure equivalent to 10 cm of mercury. The filters had previously been sterilized, and the filtrate was collected in sterile flasks. The filtrates were placed in small sterile test tubes in quantities of approximately 3 cc per tube. About 1 cc of liquid was removed aseptically from each tube for testing for virus content. The tubes with the remaining liquid were set aside to serve as checks on the presence of bacterial contaminants. Since phloem exudate is an excellent medium for bacteria, it is considered that the presence or absence of bacterial growth in these tubes furnished an accurate index as to whether the filters used were effective in holding back bacteria and other microorganisms.

TABLE 5.—*Filterability of curly top virus through various types of filters*

[20 plants were inoculated from each filtrate in each test]

Test no.	Plants infected by virus filtered as indicated				
	Check ¹	Berkefeld N	Berkefeld W	Mandler medium	Mandler fine
	Number	Number	Number	Number	Number
1.....	3	7	7	8	6
2.....	7	8	4	3	4
3.....	13	8	7	10	7
4.....	3	1	3	4	3

¹ Unfiltered.

The results of four series of tests (table 5) show that the virus in phloem exudate passed all the grades of filters used. The comparative numbers of infections do not indicate that any amount of virus measurable by this method is held by the filters. The virus apparently passes the finer grades of filters to about the same degree as it passes the coarser ones.

In further tests it was found that the virus in leaf juice, root juice, water wash of alcoholic precipitate of leaf juice, and water wash of alcoholic precipitate of phloem exudate readily passed the ordinary filters such as the Berkefeld N or Mandler medium grade.

RESISTANCE OF VIRUS TO AGING IN LIQUID MEDIUM

Numerous tests have shown that the virus in expressed beet juice retains its activity for 1 to 7 days at ordinary temperatures, but, in some instances, it may remain active for as long as 14 days. The factors responsible for these variations and those influencing inactivation are very obscure. However, since the curly top virus occurs sparsely, if at all, in parenchymatous tissue, it may well be that parenchyma cells contain materials inimical to prolonged activity of the virus. Bacterial action and products of oxidation in expressed juice are also factors which must be considered in this connection.

An experiment was planned in an attempt to separate and, if possible, evaluate these factors suspected of being important in inactivation. Badly diseased leaves from young beets were frozen and the juice expressed and centrifugalized to remove the coarser particles. The juice was then divided into four lots and treated as follows: Lot 1, tubed; lot 2, passed through a sterile Berkefeld N filter, and aseptically placed in sterile tubes; lot 3, water wash of alcoholic precipitate prepared and tubed; lot 4, water wash of alcoholic precipitate prepared, passed through a sterile Berkefeld N filter, and placed aseptically in sterile tubes.

The four lots of material were incubated at room temperature and the tubes were removed from each lot at intervals for testing the virus content. For each test a water wash of alcoholic precipitate of the contents of the tubes in lots 1 and 2 was prepared. A small amount of sugar was added to this and also to the tubes of lots 3 and 4 before the feeding of leaf hoppers. After a feeding period of approximately 4 hours the leaf hoppers were caged singly on seedling beets.

Three series of tests were made. In these there was considerable variation in infection and some variation in the time the virus remained active in the three series, but the conclusions which may be drawn from any one of these tests are in accord with those which may be drawn from the combined results of the three tests shown in table 6. These results show that in the unfiltered leaf juice the virus remained active for 7 days but not for 14 days. In the filtered leaf juice in which the virus was not subjected to the action of micro-organisms, the virus remained active for the same length of time as in the unfiltered juice. In this case micro-organisms apparently had no effect on the virus. It will be noted that the infection from filtered juice was much less than from any of the other three lots. Considerable difficulty was experienced in obtaining sufficient filtrate for tests because of the formation of a covering of colloidal material on the outside of the filter. This material probably interfered with passage of the virus and may account for the smaller number of infections.

In the unfiltered water wash of the alcoholic precipitate of leaf juice the virus was active after 14 days but not after 21 days. In the filtered wash of the alcoholic precipitate the virus was active after 28 days but not after 35 days. In the unfiltered water washings of the alcoholic precipitate micro-organisms apparently had an influence in virus inactivation. The increased tolerance to aging in both the filtered and unfiltered wash of the alcoholic precipitates as compared to the leaf juice suggests that the elimination of the materials present in leaf juice but not precipitated by alcohol considerably increases the time the virus will remain active in a liquid medium. In the leaf juice these materials may inactivate the virus before the full influence of bacterial action would be exerted and this may account for the fact that the virus remained active as long in unfiltered as in filtered juice.

TABLE 6.—Resistance of virus to aging in filtered and unfiltered leaf juice and in filtered and unfiltered wash of alcoholic precipitate of leaf juice

[60 plants were inoculated in each test]

Virus source	Plants infected after indicated number of days							
	0	1	3	7	14	21	28	35
	Number	Number	Number	Number	Number	Number	Number	Number
Leaf juice not filtered.....	33	31	28	4	0	0	0	0
Leaf juice filtered.....	13	14	12	1	0	0	0	0
Wash of precipitate not filtered.....	39	25	25	8	4	0	0	0
Wash of precipitate filtered.....	22	23	34	23	8	9	3	0

INFLUENCE OF DESICCATION ON LONGEVITY OF VIRUS

In the tests to determine the resistance of the curly top virus to desiccation several types of materials containing virus were selected. These were dried and kept at room temperature for the period of the test. Liquids for testing for the presence of the virus in the dried material were prepared at intervals by mixing the dried materials with water or with 5-percent sugar solution or by making extracts of the dried material acceptable to the beet leaf hopper. Nonvirus-liferous beet leaf hoppers were allowed to feed on these liquids and caged singly on seedling beets. The infection that resulted was accepted as a measure of the virus content of the dried material. The following materials and methods were used.

Phloem exudate.—Exudate from the cut surface of diseased beet roots was diluted 1 part exudate to 4 parts distilled water, and 6 drops of the mixture was placed on each of several capping skins attached to the bottom of cages for feeding leaf hoppers. The drops were dried at room temperature before an electric fan. A liquid for making tests of virus content was prepared by adding a drop of distilled water to each bit of dry residue. In a second test naturally occurring phloem exudate, dried to a sirupy consistency, was scraped from diseased beet petioles and placed in an open container at room temperature. At intervals a liquid for testing virus content was prepared by adding a small amount of the dry residue to 1 cc of distilled water. In a third test the alcoholic precipitate from phloem exudate was dried at room temperature and kept in an open container. Test liquids were prepared by mixing a small amount of the dry residue with 1 cc of 5-percent sugar solution.

Sugar beet plants.—Severely diseased sugar beet plants having 6 to 8 true leaves were selected from greenhouse material. Considerable exudate was present on the petioles and blades. The plants were divided into two lots. The superficial exudate was removed from the first lot by washing but was allowed to remain on

the second lot. The plants were dried in a current of air at a temperature of about 100° F. In each test for virus content six plants were pulverized in a mortar and steeped in a quantity of cold distilled water equal to approximately twice the green weight of the plants. The water was expressed and added to an equal volume of 95-percent alcohol. The precipitate resulting was thrown down by centrifugalization, washed once with 50-percent alcohol, and dried. It was then made up to a volume equal to half the volume of water added to the dried plants, with 5-percent sugar solution. This was shaken and centrifugalized, and the supernatant liquid was used as the test liquid.

Alcoholic precipitate of beet juice.—Juice was expressed from the tops and roots of diseased beets. The juice from each source was divided into lots of 5 cc each and precipitated with alcohol. The precipitate was washed once with 50-percent alcohol and dried. The dried material was preserved and tests were made at intervals by grinding each lot of dried material in 5 cc of 5-percent sugar solution, centrifugalizing, and using the supernatant liquid for feeding leaf hoppers.

Beet leaf hoppers.—Viruliferous leaf hoppers were killed by etherization, dried at about 100° F., and preserved in a vial. At regular intervals 50 leaf hoppers were removed from the vial, ground in a mortar, and mixed with distilled water. The mixture was centrifugalized and the supernatant liquid was precipitated with alcohol. The precipitate was washed once with 50-percent alcohol, dried, made up to 1 cc with 5-percent sugar solution, and centrifugalized, and the supernatant liquid was used to feed leaf hoppers.

Tests of virus content of the preparations just described were made at monthly intervals. The results of these tests are shown in table 7. As might be expected in tests of this type, there was considerable variation in the percentage of infection from month to month. These fluctuations were increased by a resistant variety of beet accidentally used in some of the tests. In spite of these fluctuations, it is evident that the virus remained active for long periods in dry material and that the nature of the medium from which the virus was taken influenced longevity.

TABLE 7 --Effect of desiccation on longevity of the curly top virus

[20 plants were inoculated in each test]

Dried material as virus source	Plants infected by virus dried for indicated number of months										
	Check	1	2	3	4	5	6	7	8	9	10
Exudate from cut surface of beet.	No. 15	No. 7	No. 16	No. 9	No. 9	No. 13	No. 4	No. 0	No. 0	No. 2	No. 2
Natural exudate	10	14	---	11	5	3	3	1	7	7	10
Alcoholic precipitate of exudate	8	7	6	4	7	3	0	0	0	0	0
Beet tops (washed free of exudate)	13	9	5	3	1	0	0	0	0	0	0
Beet tops (not washed free of exudate)	16	9	6	2	2	0	0	0	0	0	0
Alcoholic precipitate of leaf juice	6	3	2	0	0	0	0	---	---	---	---
Alcoholic precipitate of root juice	9	2	1	0	0	0	0	---	---	---	---
Beet leaf hoppers	7	3	7	4	4	2	2	---	---	---	---

There was little measurable decrease in quantity of active virus in natural phloem exudate in a period of 10 months, and a certain amount of active virus was still present in exudate from the cut surface of diseased beets after 10 months. The virus remained active for much shorter periods in all materials in which parenchyma tissue or products of parenchyma tissue occurred. Since all of the material used in these experiments was kept in the open at room temperature, the actual moisture content varied with that of the surrounding air. It is probable that the moisture content was sufficient to permit the action of any deleterious substances contained in the samples. The period of activity of the virus in preparations

containing parenchyma or products of parenchyma may have been influenced by the action of substances deleterious to the virus and contained in the parenchyma. It is significant in this connection that natural exudate, the material in which the virus retained its greatest activity, had least contact with parenchyma, since it probably escaped directly from the phloem and passed to the surface through the intercellular spaces.

EFFECT OF TEMPERATURE

LOW TEMPERATURE

Tests of the time the curly top virus will withstand temperatures below freezing have been made with the virus contained in phloem exudate as it is derived from diseased beets and also with exudate diluted 1 part exudate to 4 parts 5-percent sugar solution. Small glass tubes were filled with these materials, sealed at both ends, and placed in a refrigerator held at approximately -6°C . The tubes were removed and their contents tested at monthly intervals, in the usual way, by means of nonviruliferous beet leaf hoppers. The results of these tests (table 8) indicate that the virus retains its activity for at least as long as 18 months.

TABLE 8.—*Effect of prolonged periods of freezing on virus activity*

[20 plants were inoculated in each test]

Virus-bearing material	Plants infected by virus frozen for indicated number of months											
	Check	1	2	3	4	6	7	8	9	11	12	18
Phloem exudate not diluted	No. 6	No. 16	No. 12	No. 14	No. 14	No. 16	No. 12	No. 8	No. 9	No. 4	No. 6	No. 3
Phloem exudate diluted 1 : 4	8	15	13	13	14	16	12	8	14	2	6	3

During the course of other experiments it was repeatedly found that the virus retained its activity for prolonged periods in a variety of frozen materials contained in test tubes, 25-cc flasks, and open beakers. No difficulty has been experienced in obtaining active virus from materials such as exudate, water wash of alcoholic precipitate of exudate, or water wash of alcoholic precipitate of beet juice, after a storage period of several months. Advantage has been taken of this fact to collect relatively large quantities of virus-containing substances for subsequent experimental work. These stored materials have afforded a more uniform source of virus for repetition of experimental work and comparison of results than otherwise would have been available.

ALTERNATE FREEZING AND THAWING

Attempts were made to determine the effect of alternate freezing and thawing on the virus contained in phloem exudate and also on the virus contained in the water wash of the alcoholic precipitate of leaf juice. These materials were placed in small test tubes in 1-cc quantities. The tubes were placed in the freezing compartment of a refrigerator held at approximately -6°C . The tubes were removed

daily and the contents thawed at room temperature and returned to the refrigerator. Tubes which served as checks were kept frozen continuously until tested. Tests of virus content were made at weekly intervals. When the experiment was terminated at the end of 3 weeks, there was no measurable decrease in virus concentration in either type of material and apparently the quantity of active virus is not measurably diminished by such a treatment.

HIGH TEMPERATURES

In order to determine temperatures at which the virus would remain active, tests were made with phloem exudate diluted 1 part exudate to 4 parts 5-percent sugar solution. Thin-walled glass tubes with a maximum diameter of about 2 mm were filled with this diluted exudate and sealed at both ends. The tubes were subjected to the desired temperature ($\pm 0.5^\circ \text{C}.$) in a water bath for 10 minutes. After treatment, the tubes were removed and the contents were made available to nonviruliferous leaf hoppers which were later caged singly on seedling beets.

Table 9 shows the results of five series of tests. Under the conditions of these tests the thermal inactivation point of the curly top virus lies between 75° and $80^\circ \text{C}.$ Perhaps the most interesting feature of these results is the rather small variation in temperature at which inactivation occurred in the different lots of exudate. In the four tests in which 1° temperature intervals were used, inactivation occurred at 80° , 79° , 76° , and 78° , respectively.

Tests similar to those just described were made in which the time of exposure to high temperatures was increased to 1 hour. In these tests leaf hoppers obtained virus from material subjected to 60° , 65° , and $70^\circ \text{C}.$ but not from that subjected to 75° and 80° .

TABLE 9. *Effect on the sugar beet curly top virus of 10 minutes' exposure to various temperatures*

[20 plants were inoculated from exudate treated at each temperature in each test]

Test no.	Plants infected by virus exposed for 10 minutes to indicated temperature ($^\circ \text{C}.$)									
	Check	60	65	70	75	76	77	78	79	80
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
1.....	7	9	11	8	7	14	13	11	3	0
2.....	16	19	14	13	11	3	0
3.....	12	11	8	10	2	0	0
4.....	15	3	0	0	0	0	0
5.....	5	4	6	1	0	0	0

ATTENUATION OF VIRUS

In the thermal-inactivation-point tests just described it was observed that the plants infected by virus treated for 10 minutes at 76° to $79^\circ \text{C}.$ grew well and produced relatively mild symptoms of disease. The leaves showed a medium amount of curling and the petioles were only slightly or not at all shortened. In contrast, the plants infected by the untreated virus were severely affected and remained small. The leaves were badly curled, the petioles were short, and several of the plants died before the experiments were terminated. Representative differences in growth are shown in figure 5.

On three series of plants inoculated with virus subjected to the differential temperatures, records of the incubation period of the disease, severity of symptoms, and dry weight of the plants after 6 to 8 weeks were made. These data for one experiment are shown in table 10. The results are similar to results obtained in the other two experiments except that in one experiment the heat treatments did not so markedly influence the period of incubation of the disease. It will be noted that the incubation period of the disease and the dry weight of affected plants tended to increase and the severity of symptoms tended to decrease as the temperature to which the virus was subjected was increased. These results show that the virus was attenuated to a considerable degree by the heat treatments and that attenuation occurs after a very short period of exposure.

In 2 of the 3 experiments virus was transferred to healthy plants from the checks and from representative plants affected by virus from

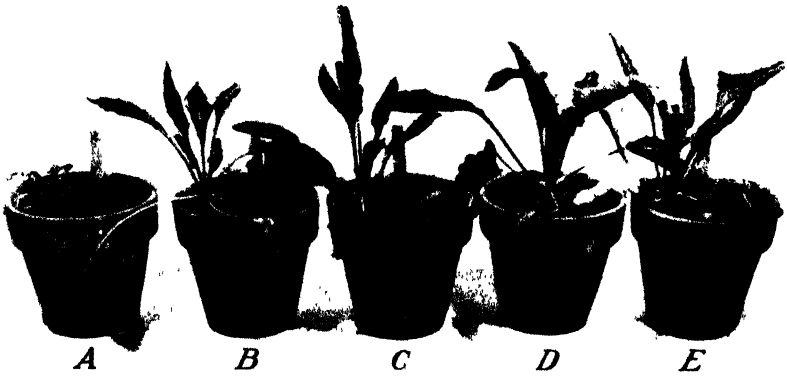


FIGURE 5.—Effect of 10-minute heat treatment on curly top symptoms produced in host plants. The virus with which these plants were inoculated was treated for 10 minutes at the following temperatures: A, Untreated, B, 76° C.; C, 77°; D, 78°; E, 79°.

different heat treatments. In one test the passage of the virus was continued through four lots of healthy plants. Relative differences in severity of disease were maintained throughout these tests, indicating that the virus in its attenuated form was relatively stable.

TABLE 10.—Attenuation of the curly-top virus by heat

Temperature of 10-minute treatment (° C.)	Plants inoculated	Plants infected	Average incubation period	Average severity ¹	Average weight of diseased plants
	Number	Number	Days	Grade	Grams
(2).....	19	16	10.2	4.4	0.26
75.....	20	19	11.3	3.3	.47
76.....	20	14	12.5	1.8	.71
77.....	20	13	13.1	1.2	.57
78.....	20	11	18.0	1.8	.79
79.....	20	3	19.3	1.6	1.13
80.....	20	0			

¹ Five grades of severity were used, plants in grade 1 being the least severely affected and those in grade 5 being the most severely affected.
² Virus untreated.

The rapidity with which attenuation was effected in the experiments just described is in striking contrast to results obtained in attenuation of other viruses at high temperatures. Johnson (11) reported attenuation of tobacco mosaic virus in tobacco plants exposed to a temperature of 35° to 37° C. for 10 days. Cucumber mosaic virus in tobacco plants was attenuated after a 10-day exposure to 37°. The question arises as to whether the curly top virus might be attenuated also at temperatures below 76° to 79° if the period of exposure were increased.

EFFECT OF HYDROGEN-ION CONCENTRATION

Tests were made to determine the effect of hydrogen-ion concentration on the virus contained in phloem exudate. Solutions buffered at approximately even pH units from 2.0 to 10.0, inclusive, were prepared according to the method of Clark (6). Measured quantities of phloem exudate were added to the buffered solutions in the proportion of 1 part exudate to 4 parts buffer. The mixtures were thoroughly shaken and allowed to stand for 2 hours. At the expiration of this time a portion of the liquid from each mixture was removed for a hydrogen-ion determination and the remainder was placed on a membrane to be fed on by nonviruliferous beet leaf hoppers. The hydrogen-ion determinations of the mixtures of the virus with buffers ranging in reaction from pH 2.0 to 7.0 were made by means of the quinhydrone electrode. Determinations on solutions of higher pH value were made by means of the glass electrode. The leaf hoppers were allowed a feeding period of 4 hours. Thus the virus was subjected to the reaction of the medium for a minimum period of 2 hours and a maximum period of 6 hours, the actual time depending on the time during the feeding period at which it was taken up by the leaf hoppers. At the end of the 4-hour feeding period the leaf hoppers exposed to the 2 liquids of greatest acidity and those exposed to the 2 liquids of greatest alkalinity were noticeably weaker than the individuals of other lots. The results of three series of tests are shown summarized in experiment 1 of table 11.

TABLE 11.—Effect of a 2-hour treatment of virus in buffer solutions of different hydrogen-ion concentrations

Experiment 1 ¹		Experiment 2 ²	
Reaction of buffer solution (pH)	Plants infected	Reaction of buffer solution (pH)	Plants infected
	Number		Number
2.4	0	2.7	0
2.9	0	3.4	2
4.0	1	4.4	21
5.0	5	5.1	15
5.9	6	6.1	19
6.8	20	7.0	21
7.6	20	7.4	4
8.4	31	8.4	14
Check ³	21	9.1	9
		Check	16

¹ 60 plants inoculated.

² 40 plants inoculated.

Additional tests made were similar to those of experiment 1 except that the exudate, after standing in contact with the buffer for 2 hours, was adjusted to a reaction of approximately pH 6.0 and precipitated with alcohol, and that a water suspension of the precipitates was prepared for the leaf hoppers. This provided for a definite exposure for 2 hours to the indicated pH and in each case supplied a uniform medium for the leaf hoppers, thus eliminating the unfavorable liquids of the more acid and the more alkaline treatments of the first tests. The results are shown in experiment 2 of table 11.

The results of these two experiments show a certain amount of unexplained variation in the number of plants infected at the different hydrogen-ion treatments. They agree, however, in that no virus was obtained from liquids in which the virus had been subjected to a reaction of pH 2.9 or lower. Infection from mixtures in which the exudate with the respective buffers was fed direct to the leaf hopper increased with the rise in alkalinity, despite the fact that the mixtures of higher pH values were obviously unfavorable as food for the leaf hopper. The reasons for the erratic results from the treatments at pH values of 7.4 and above in experiment 2 have not been determined definitely. As shown in the amount of infection at corresponding pH values in the tests of experiment 1, the virus is not inactivated at these reactions. It seems probable that the explanation may be found in an incomplete precipitation of the virus or in a close union of virus with the precipitate which prevented its being acquired by the leaf hoppers in quantities representative of its real concentration.

EFFECT OF CHEMICAL REAGENTS

ACETONE AND ALCOHOL

Tests were made with acetone and ethyl alcohol to determine the effect of different concentrations of these reagents on the virus. In these tests phloem exudate was made up to the desired concentration of reagent by the addition of acetone or absolute alcohol direct to the exudate. In the pure acetone and absolute alcohol treatments the exudate was dried before the reagent was added. The tubes containing the exudate in various concentrations of acetone and alcohol were corked, shaken well, and set aside for a period of 2 hours. At the expiration of this time the contents of the tubes were poured into watch glasses and evaporated to dryness. The residues were taken up in a volume of 5-percent sugar solution equal to five times the original volume of exudate. Nonviruliferous leaf hoppers were given access to this material for a period of 4 to 5 hours and then caged singly on seedling beets. The infection obtained was taken as a measure of the virus content of the medium on which the leaf hoppers fed.

The results of these tests (table 12) indicate that the curly top virus has a very great degree of tolerance to high concentrations of both acetone and alcohol. Of all the treatments used, only absolute alcohol seemed to have any deleterious effect on the virus. Even in absolute alcohol it was not completely inactivated by a 2-hour exposure.

TABLE 12.—*Effect of a 2-hour treatment with different concentrations of alcohol and acetone on the virus in phloem exudate*

[20 plants were inoculated at each concentration in each test]

Reagent and test no.	Plants infected by virus treated with reagent of indicated concentration (percent by volume)										
	Check	10	20	30	40	50	60	70	80	90	100
Alcohol:	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
1.....	13	13	17	16	16	13	15	16	16	15	2
2.....	8	12	16	13	15	14	17	17	13	17	2
3.....	8	7	9	9	5	5	13	13	11	8	4
Acetone:											
4.....	15		16		20		13		16		14
5.....	9										7

After it was found that the virus retained its activity after a 2-hour treatment with the various concentrations of acetone and alcohol used in the experiment just described, tests were made to determine how long the virus would remain active in different concentrations of these two reagents. Measured quantities of exudate were placed in small test tubes. Sufficient acetone or alcohol was added to make concentrations of 25, 50, and 75 percent of these reagents in the resulting mixtures. Tests of active virus content were made at intervals by taking measured quantities of material from the tubes holding the different concentrations, and evaporating to dryness. As in previous experiments, the residues were taken up in sugar solution and tests for virus activity were made with nonviruliferous leaf hoppers.

A second test was made with beet juice in 25-, 50-, and 75-percent alcohol. The mixtures were kept in stoppered flasks at room temperature. In making the tests for virus, the contents of the flasks were shaken well, after which enough liquid was poured out to correspond to 1 cc of the juice. This was centrifugalized and the supernatant liquid discarded. The precipitate was washed once with 50-percent alcohol, dried, mixed with 1 cc of 5-percent sugar solution, and centrifugalized, and the supernatant liquid was used for testing.

TABLE 13.—*Longevity of virus in phloem exudate and beet leaf juice in different concentrations of alcohol and acetone*

[20 plants were inoculated in each test]

Virus source and reagent	Con- centration of reagent in mixture	Plants infected by virus treated for indicated number of days								
		0	1	3	7	14	21	28	35	56
Phloem exudate:	Per- cent	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber
Alcohol.....	25	10	6	8	8	7	6	0	2	0
	50	6	9	8	10	7	1	5	3	3
	75	5	8	8	4	9	8	4	2	1
Acetone.....	25	12	11	9	5	5	3	0	1	0
	50	10	6	11	6	5	6	1	0	0
	75	9	9	5	7	0	3	0	1	2
Beet leaf juice:										
Alcohol.....	25	11	8	11	0	0	0	0	0	-----
	50	13	9	9	2	0	0	0	0	-----
	75	13	2	2	1	0	0	0	0	-----
Beet leaf juice.....	-----	8	6	10	0	0	0	0	0	-----

The results of these tests are shown in table 13. The longevity of the virus was apparently not influenced by the concentration of alcohol regardless of whether phloem exudate or beet juice was the original source of the virus. However, longevity of the virus was markedly influenced by the kind of plant material from which the virus was derived. Where phloem exudate served as the source of virus, active virus was present after 56 days in two of the alcohol preparations. Where beet juice was present, the virus was inactivated in 14 days, a period corresponding to the period of inactivation in beet juice without alcohol. This is further evidence indicating the presence of an inactivating substance in beet juice not present or present only in low concentrations in phloem exudate. Several tests have indicated that when alcohol or acetone is added to phloem exudate from beet the virus remains active 2 to 4 weeks longer than in similar material to which nothing is added. This indicates not only that acetone and alcohol fail to exert any appreciable deleterious effect on the virus but that they may function to protect the virus somewhat from the full effects of factors which, in the absence of acetone or alcohol, normally cause fairly rapid inactivation.

The resistance of virus contained in phloem exudate to the lethal action of alcohol is apparently rather marked as compared to other plant viruses. Fajardo (9) found that the virus of bean mosaic loses its activity in 50-percent alcohol in less than 5 minutes. Allard (1) showed that the tobacco mosaic virus is inactivated very quickly in concentrations of alcohol greater than 50 to 55 percent and that it remains active for less than 30 minutes in a concentration of 80-percent alcohol. Allard also found that with tobacco mosaic virus alcohol was more destructive than acetone.

GERMICIDES

Such common germicides as bichloride of mercury, copper sulphate, formaldehyde, and carbolic acid were used in a series of tests to determine their effects on the virus. Since the beet leaf hopper must be used to transfer the virus to the test plants, it was necessary after treatment to separate the virus from the reagents before passing it through the leaf hoppers. It was found that with all of these reagents except formaldehyde this separation could be made by removing the virus in the precipitate formed upon the addition of alcohol. Measured quantities of phloem exudate were placed in centrifuge tubes. An equal volume of reagent made up to twice the concentration desired in the final mixture was added. The mixture was thoroughly agitated and allowed to stand at room temperature for 2 hours. At the end of this time, except in tests where formaldehyde was used, 95-percent alcohol was added to each tube in amount equal to the volume of liquid already in the tube. The tubes were shaken and allowed to stand until a precipitate was formed. This usually required from 5 to 10 minutes. The precipitate was thrown down in a centrifuge and the supernatant liquid, containing most of the reagent, was discarded. The residue was washed once with 50-percent alcohol to remove additional quantities of reagent. It was then dried to remove the alcohol, after which it was made up to five times the original volume of exudate with 5-percent sugar solution, and the tests were made for virus content as in previous experiments. In the

formaldehyde tests the exudate, after a 2-hour treatment with formaldehyde, was evaporated to dryness at room temperature and then taken up in 5-percent sugar solution.

TABLE 14.—Effect of a 2-hour treatment of virus in phloem exudate with different concentrations of germicides

[20 plants were inoculated at each concentration in each test]

Reagent and test no	Plants infected by virus treated with reagent of in limited concentration								
	1:25	1:50	1:100	1:200	1:300	1:400	1:500	1:1,000	Check
Copper sulphate	Number	Number	Number	Number	Number	Number	Number	Number	Number
1		0	0	0			13	14	16
2	0	0	0	2		15	14	11	12
3	0	0	0	0	1	0	6	16	12
Bichloride of mercury								13	15
1		7	4	7			10	11	18
2	0	1	2	1	1	3	2	1	5
3									
Formaldehyde								11	13
1		0	0	1			3	6	5
2			1	1	5	4	11	4	7
3	0	0	0	0	2	6	16	17	16
Carbolic acid								4	5
1	0	1	1	7			6		
2	4	8	10	9	16	13	13		14

Results summarized in table 14 show that the virus is quite resistant to the action of these germicides. Copper sulphate was more effective in inactivation than any of the other materials tested, no infection being obtained from exudate treated for 2 hours in a concentration of 1:100. The leaf hoppers were able to pick up virus from exudate subjected to a 2-hour treatment with carbolic acid (1:25), bichloride of mercury (1:50), and formaldehyde (1:100), although infection was reduced at these concentrations. It is probable that carbolic acid and bichloride of mercury at a concentration of 1:25 had even less effect on the virus than is indicated by the number of infected plants. From the 1:25 concentrations of both carbolic acid and bichloride of mercury it was found difficult to remove enough of the germicide to avoid an effect on the leaf hoppers. Feeding was poor and the leaf hoppers were weak when they were removed from the medium and placed on plants, consequently it is improbable that the insects were as effective in picking up and transmitting the virus as they would have been had the medium on which they fed been more favorable.

INACTIVATION OF VIRUS BY PLANT JUICES

Severin and Freitag (13) showed that the expressed juice from Mammoth sweet corn mixed with juice from diseased beets inactivated the curly top virus in 2 hours. Results of tests described in this paper show that the virus in filtered beet juice and in beet juice to which alcohol was added was inactivated in 1 to 2 weeks, whereas, in filtered phloem exudate and in phloem exudate to which alcohol was added, the virus remained active 4 to 7 weeks. This evidence indicates that beet juice contains substances capable of inactivating the virus, though these inactivating substances are evidently not so effective in causing inactivation as those found in the juice of sweet corn.

To test further the effect of plant juices in inactivation of the curly top virus a number of species of plants ranging in susceptibility to curly top from very susceptible to very resistant have been selected. These tests were made with juice from Australian saltbush (*Atriplex semibaccata* R. Br.), nettle-leaved goosefoot (*Chenopodium murale* L.), tree tobacco (*Nicotiana glauca* Graham), cultivated tobacco (*N. tabacum* L.), yellow sorrel (*Oxalis corniculata* L.), tomato (*Lycopersicon esculentum* Mill.), alfileria (*Erodium cicutarium* (L.) L'Her.), chickweed (*Stellaria media* (L.) Cyr.), and susceptible and resistant beets (*Beta vulgaris* L.). Of these species Australian saltbush, nettle-leaved goosefoot, and tree tobacco are very resistant, and alfileria, chickweed, and the susceptible variety of beet are very susceptible. The other species are somewhat intermediate in susceptibility.

In making the tests, juice from the plants to be tested was mixed with juice from diseased beets in the proportion of 4 parts test juice to 1 part juice from diseased beets. The mixtures were placed in stoppered flasks and allowed to stand at room temperature. At intervals, 5 cc of each mixture was removed, added to 5 cc of 95-percent alcohol, and centrifugalized, and the supernatant liquid was discarded. The precipitate was washed once with 50-percent alcohol, dried, mixed with 1 cc of 5-percent sugar solution, and centrifugalized, and the supernatant liquid was used for testing for virus.

TABLE 15.—Inactivation of the curly top virus by juices from different species of plants susceptible to curly top

Source of juice	Plants inoculated	Plants infected by mixture of indicated age					
		Check ¹	2 hours	1 day	3 days	7 days	14 days
	Number	Number	Number	Number	Number	Number	Number
<i>Atriplex semibaccata</i>	60	5	6	1	0	0	0
<i>Chenopodium murale</i>	60	13	13	25	23	13	1
<i>Nicotiana glauca</i>	40	21	17	16	14	5	0
<i>Nicotiana tabacum</i>	60	37	31	24	10	3	1
<i>Oxalis corniculata</i> (pH 2.3-2.5).....	60	6	0	0	0	0	0
<i>Oxalis corniculata</i> (pH 6.8-7.2).....	60	13	2	6	0	0	0
<i>Lycopersicon esculentum</i>	60	20	8	6	7	1	0
<i>Erodium cicutarium</i>	60	17	5	13	4	0	0
<i>Stellaria media</i>	60	9	12	21	4	0	0
<i>Beta vulgaris</i> (resistant).....	60	21	23	20	26	13	1
<i>Beta vulgaris</i> (susceptible).....	100	32	30	43	48	22	1

¹ Precipitation was made immediately after the juice from the test plant was mixed with juice from diseased beets.

The results of this series of tests are shown in table 15. Of the three most resistant plants the juice from Australian saltbush quickly inactivated the virus, whereas juice from nettle-leaved goosefoot and tree tobacco in general had no more effect than juice from beet. Of the three species probably most susceptible, the juice from alfileria and chickweed inactivated the virus in 7 days and juice from beet did not inactivate the virus in 14 days. These results are more striking in view of the fact that tree tobacco is a symptomless virus carrier (2), nettle-leaved goosefoot attenuates the virus (3), and chickweed restores attenuated virus to its maximum virulence (12).

Juice from yellow sorrel was the most effective of the juices in producing inactivation. Results in table 15 show that no virus was obtained from juice of this plant after 2 hours, and results of other tests not shown in table 15 indicate that the virus was inactivated in 30 minutes.

Juice from tomato and alfalfa produced more rapid inactivation than juice from beet. Apparently juice from resistant beets is no more effective in producing inactivation than that from susceptible beets. It is obvious from these results that expressed juice from different species of plants varies greatly in its ability to inactivate the virus and that this variation is not correlated with resistance.

The question naturally arises as to how much of this apparent inactivation is due to an irreversible change in the virus itself and how much is due to an adsorption of virus by colloidal particles in such a way as to prevent its acquisition by leaf hoppers. It seems probable that juices from different species of plants vary in respect to the readiness with which virus is released from their alcoholic precipitates. For example, as compared with the addition of juice from healthy beets to the original virus-carrying medium, the addition of juice from tobacco increased and the addition of juice from chickweed and alfalfa decreased the amount of virus recovered from the alcoholic precipitates. Since this result was evident in the short-time exposures and since the virus remained active in these mixtures for 3 or more days, it seems probable that much of the variation in infection from preparations made during the first 2 hours of aging of the juices may be the result of variations in completeness of release of the virus from colloidal material into a supernatant liquid. However, the rapid inactivation by juice from yellow sorrel and the more gradual loss of virus in other juices indicates that there are factors present that are capable of causing complete and permanent inactivation.

The source of the inactivating materials present in plant juice has not been determined definitely. Severin and Freitag (13) found that virus apparently remained active for a longer time in beet juice from which air was partially excluded than in juice exposed to the air, the implication being that virus is inactivated by products of oxidation in expressed juice. However, the rapid inactivation by certain plant juices indicates that there are substances other than products of oxidation that are destructive to the virus. Also the evidence (2) indicating the occurrence of little or no virus in parenchyma tissues points to the occurrence of substances in parenchymatous cells that either inactivate the virus or prevent its multiplication. It seems probable that at least a large share of the inactivating substances present in plant juices are derived from living parenchyma cells.

If the inactivating substances are derived from parenchyma, the lack of correlation between the apparent concentration of inactivating substances in expressed juice and resistance does not necessarily eliminate the inactivating substances as factors in resistance. Since the virus is concentrated in the phloem, it is probably not greatly influenced by inactivating materials that occur only in the parenchyma. However, if inactivating substances pass from the parenchyma into the phloem their effect on the virus and perhaps their importance in resistance may depend on the concentration reached in the phloem. Under such conditions the permeability of the cells surrounding the phloem to inactivating substances might be more important in determining resistance than the total concentration of inactivating materials in the plant.

The chemical nature of substances in plant juices responsible for virus inactivation, especially those present in the juice of yellow sorrel and Australian saltbush, offer an interesting field for investigation.

At present very little is known about the character of these substances and any statements that are made on this subject must necessarily fall more or less in the realm of speculation. However, since the virus is quickly inactivated at a reaction of pH 2.9 and lower and is apparently somewhat sensitive to acid reactions, it is suggested that acidity of the expressed juice may have an inactivating influence in some instances. This would apply especially to yellow sorrel, since the reaction of expressed juice from this plant ranges between pH 2.0 and 2.3 and when mixed with beet juice in the proportion of 4 parts yellow sorrel juice to 1 part beet juice, the reaction of the mixture ranges between pH 2.3 and 2.5. However, when this juice was adjusted to a reaction of pH 6.8 to 7.0 it was still capable of inactivating the virus, though inactivation was much less rapid than when the reaction was not adjusted. It seems probable that expressed yellow sorrel juice contains two factors, each capable of inactivating the virus; the more effective of these factors is acidity and the less effective is the inactivating substances remaining when acids are neutralized.

Substances not acid in character are probably responsible for inactivation in juices from the remainder of the plants tested. With the exception of juice from alfalfa, which has a reaction of pH 5.2 to 5.6, the juices from these plants have about the same degree of acidity as beet juice, ranging from pH 5.8 to 6.8. It does not seem probable that this range of acidity could account for the wide range in ability to inactivate the virus, especially since there is no correlation between acidity and rate of inactivation.

DISCUSSION

The physical and chemical properties of the curly top virus, so far as they have been determined, are similar in many respects to the properties that are considered typical of plant viruses as a group. However, the curly top virus has certain characteristics that are specific. Among the characteristics that serve to distinguish it from other viruses may be mentioned an extreme resistance to the deleterious action of alcohol and acetone and an activity in a range of hydrogen-ion concentrations that extends far toward the alkaline side of the scale.

The available information affords no definite clue as to the reason for prolonged periods of activity of the curly top virus in alcohol and acetone. The elucidation of this problem must await a more complete understanding of the influence on the virus of certain chemical and environmental factors. However, certain evidence is available that may have a bearing on the reaction of the virus to hydrogen-ion concentration. As compared with other viruses that have been studied in this connection, a greater sensitivity to acid reactions and a greater tolerance to alkaline reactions might be expected on the basis of the relation of the virus to the plant and to the insect vector.

In the beet plant the virus is rather definitely known to be located in the phloem. The tissues or fluids associated with the retention of the virus by the beet leaf hopper have not been determined completely but it is quite evident that the virus passes from the leaf hopper to the plant through the medium of the saliva. Evidence

not yet published indicates that the virus is present also in the body fluid. In view of this evidence and of the fact that Storey (16) has shown that the virus of maize streak occurs and multiplies in the body fluid of its leaf hopper vector, *Balclutha mbila* Naude, it seems probable that the body fluid may be the chief reservoir of the curly top virus in the beet leaf hopper.

The hydrogen-ion concentration of the various tissues and fluids of the plant and insect vector in which virus occurs has been determined within certain limits. Natural exudate on beet petioles and small drops of exudate that appear immediately above the ends of the veins on the cut surface of beet roots are alkaline in reaction. Physiologists have shown for a number of plants that the phloem content is alkaline, and it is safe to assume that the reaction of the phloem content of beet is well above pH 7.0. Fife (10) has shown that the salivary secretions in the beet leaf hopper are distinctly alkaline. Determinations⁴ made on the body fluid have given values ranging from pH 7.0 to 7.6. Thus, with the possible exception of a period in the alimentary tract of the insect following ingestion of plant substance taken up in feeding, the virus normally exists in a medium alkaline in reaction. The virus apparently has a greater tolerance to alkaline compounds than that possessed by viruses that occur naturally and increase in the acid cell sap of parenchymatous tissue.

Certain results of the studies on properties may be interpreted as having a bearing on the relation of the virus to the sugar beet plant. For example, the results of the studies on dilution clearly indicate that less virus is available from expressed beet juice than is available from phloem exudate. This is shown both by the number of plants infected and by the maximum dilutions from which virus was obtained. These results probably reflect a difference in initial concentration of virus in the two types of material. Considering the experiments in which only one leaf hopper was used on each test plant, the maximum dilutions from which virus was obtained were 1:1,000 for phloem exudate and 1:300 for expressed beet juice. Thus it might be reasoned that the concentration of virus in phloem exudate is more than three times that in beet juice. On the basis of infection obtained from the different dilutions of material tested, assuming that 0.1 g of leaf hoppers is equal in volume to 0.1 cc, the virus content of the beet leaf hopper is slightly less than that of phloem exudate but considerably greater than that of beet juice. The results with different dilutions of phloem exudate and beet juice constitute additional evidence to support the concept that virus in the beet plant is concentrated in the phloem. The virus found in expressed juice may be derived from material that has passed from the phloem into the intercellular spaces of surrounding tissues or from phloem content that escapes and becomes mixed with sap from other tissues during the process of extraction.

Since so little is known regarding the factors responsible for the close association of the curly top virus with the phloem, and its partial or practically complete restriction to this tissue, certain of the results of these studies are of interest as having a bearing on this subject. In any limitation of virus to phloem there are two possibilities that must be considered: (1) Tissues that surround the phloem

⁴ These determinations were made in collaboration with J. M. Eife, biochemist, Division of Sugar Plant Investigations, Bureau of Plant Industry. Details of this work have not been published.

may tend to prevent passage of virus into or out of the phloem, and (2) virus that gains entrance to parenchymatous tissues may remain active but fail to multiply or it may be inactivated. The permeability factor may be important in limiting the quantity of virus that gains entrance to parenchyma, but this factor alone can hardly account for the virus distribution and concentration that has been shown to occur in different tissues of the plant. Virus probably gains entrance to parenchyma in several ways. It is possible that it passes directly from the phloem into the surrounding parenchyma cells, and it is undoubtedly introduced into parenchyma cells by artificial inoculation and by leaf hopper feeding. However, there is no evidence that increase of the virus in parenchyma follows its introduction by any of these methods. In view of these facts it is logical to conclude that parenchyma tissue is unfavorable for virus multiplication and that certain constituents of parenchyma cells may be capable of inactivating the virus.

Results of experiments with expressed plant juices show that substances capable of causing rapid inactivation occur in the juice of yellow sorrel and that substances capable of causing less rapid inactivation occur in the juice of beet and several other plants. If these inactivating substances occur also in the sap of living cells, as seems probable, they may be the factors chiefly responsible for the partial or practically complete restriction of the virus to the phloem. If their behavior in the living cell is comparable to their behavior in expressed juice, their effectiveness would vary with the species of plant involved. Practically complete restriction of virus to the phloem might be expected in yellow sorrel and Australian saltbush, and less complete restriction might be expected in such plants as beet and tobacco.

SUMMARY AND CONCLUSIONS

Because of the difficulty of obtaining infection by artificial inoculation and the necessity of using the beet leaf hopper for determining the presence of virus in any particular medium, property studies of the curly top virus have required the development of a technic for utilizing the vector for transmission experiments, which has not been necessary in similar studies with viruses that more readily produce infection after artificial inoculation. The first material from which artificially fed leaf hoppers were induced to acquire virus was beet juice. However, this proved to be either toxic or unacceptable to the beet leaf hopper and is not a very satisfactory material for use in property studies. Two types of material that have a relatively high virus content and are more acceptable to the beet leaf hopper have been found. Of these two materials the more satisfactory is the phloem content of diseased beet plants. This can be obtained in small quantities as natural exudate from the beet petioles and leaves and in larger quantities from the cut surface of diseased beet roots. The second type of material was prepared by separating the virus in beet juice from the materials toxic or unacceptable to the beet leaf hopper. In effecting this separation the precipitate produced upon the addition of alcohol is thrown down by centrifugalization, washed once with 50-percent alcohol, dried, made up to the original volume of juice with 5-percent sugar solution, and centrifugalized. The supernatant liquid of the second centrifugalization contains considerable virus and is a favorable food for the beet leaf hopper.

This method of preparing a virus-containing medium can be used with phloem exudate, suspensions of crushed beet leaf hoppers, or other materials containing proteins. Acetone may be used instead of alcohol without appreciably influencing the result. The method is valuable in separating virus from materials that are toxic or unacceptable to the beet leaf hopper, especially in experiments involving the treatment of the virus with various chemical compounds.

The removal of the virus from beet juice and phloem exudate by the precipitate formed upon the addition of alcohol is practically complete. The release of the virus from the precipitate upon the addition of 5-percent sugar solution is only partial, since considerable virus was obtained from each of several successive washings of precipitate from phloem exudate and from three successive washings of precipitate from beet juice.

When the materials just described were used as sources of virus it was found that virus concentration in phloem exudate was considerably greater than that in beet juice and somewhat greater than that in the beet leaf hopper. The dilution of phloem exudate from which virus was recovered apparently depended somewhat on the number of artificially fed leaf hoppers used to inoculate each seedling beet. Virus was recovered from dilutions of 1 : 1,000 in experiments in which 1 artificially fed leaf hopper was placed on each plant and from dilutions of 1 : 20,000 in experiments in which 10 leaf hoppers were placed on each plant.

The common filters, such as the Berkefeld V, N, and W and the Mandler medium and fine grades, do not remove the virus from phloem exudate, water suspension of alcoholic precipitate of leaf or root juice, or from beet leaf juice. With the exception of leaf juice, the recoverable virus in the liquids after passing through these filters was about as great as in the unfiltered original liquid.

The resistance of the virus to aging in a liquid medium depends considerably on the medium in which the virus is preserved and perhaps somewhat on the presence or absence of micro-organisms. In filtered and unfiltered beet leaf juice virus was recovered after 7 days. It was recovered from unfiltered water wash of alcoholic precipitate of leaf juice after 14 days and from filtered water wash of alcoholic precipitate of leaf juice after 28 days.

The resistance of the virus to desiccation also depends somewhat on the medium in which it is kept. Virus remained active 10 months in dried phloem exudate, 5 months in alcoholic precipitate of phloem exudate, 4 months in dried beet tissue, 2 months in alcoholic precipitate of beet leaf juice and beet root juice, and 6 months in dried beet leaf hoppers.

Phloem exudate subjected to a temperature below freezing for a period of 18 months showed no great loss in virus content. Daily alternate freezing and thawing for a period of 3 weeks did not appreciably lessen the virus content of diluted phloem exudate or that of the water wash of the alcoholic precipitate of leaf juice. The thermal inactivation point of the virus lies between 75° and 80° C. A 10-minute exposure to a temperature of 76° to 79° attenuated the virus.

No virus was recovered from liquids having a pH value of 2.9 or lower. Alkaline reaction of the medium at least as high as pH 9.1 did not inactivate the virus in 2 hours and, in most instances, the alkaline reactions probably had little or no deleterious influence.

Evidence is presented indicating that the virus normally occurs in an alkaline medium in both the plant and insect vector. This may account in part for the resistance of the virus to inactivation by alkaline compounds.

A 2-hour treatment with 90-percent alcohol and with lower concentrations had no appreciable effect on the virus. Absolute alcohol reduced but did not destroy activity in a 2-hour period. A 2-hour treatment with acetone had no apparent effect at any concentration. Virus was active after 56 days in 50- and 75-percent alcohol and in 75-percent acetone, although activity had been considerably decreased. The virus also exhibits considerable resistance to the lethal action of a number of common disinfectants, active virus being recovered from copper sulphate (1:200), bichloride of mercury (1:50), formaldehyde (1:100), and carbolic acid (1:25).

The expressed juice from beet and a number of other species of plants is able to cause inactivation of the curly top virus in periods ranging from 30 minutes to more than 14 days, depending on the species of plant from which the juice is derived. The time required for inactivation by juice from different species of plants apparently is not correlated with the degree of resistance of the species. Since the curly top virus is believed to be more or less closely restricted to the phloem, it is suggested that if the inactivating substances in expressed juice are derived directly from the plant, as seems probable, they occur in the parenchymatous tissues. If they have any effect in producing resistance this effect is probably governed by the degree to which they are able to diffuse into the phloem. Inactivating substances of the parenchyma may function in limiting virus to the phloem tissue.

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PRODUCTIVITY OF THE CAMPHOR SCALE AND THE BIOLOGY OF ITS EGG AND CRAWLER STAGES¹

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INTRODUCTION

The eggs of the camphor scale (*Pseudaulnobia duplex* Ckll.) are laid beneath the scale covering of the mother, and there they complete their development. Upon hatching they are known as "crawlers." After an indeterminate time the crawlers emerge and crawl about over the host plant until they settle and begin feeding. The present studies are concerned with this critical phase of the life history, during which the maximum abundance and distribution of the succeeding generation are determined. The later development of the scale is discussed in another paper.²

PRODUCTIVITY

DETERMINED FROM EMERGENCE IN THE LABORATORY

The productivity of the camphor scale cannot be studied directly without removing the scale covering that conceals the eggs, but that of undisturbed females can be estimated indirectly. Small potted camphor-trees and Satsuma orange trees on which there were one or more mature females were examined periodically, and all crawlers and newly settled young were recorded and removed. In some cases the observations were as frequent as every 3 hours during the day, in others at intervals of 2 or 3 days; hence these records exclude a variable number of crawlers that emerged and died between observations. Furthermore, the natural variability in productivity may have been augmented by differences in the potted plants and in their position in the insectary. For these reasons the plants on which there were more than an average number of offspring per female may sometimes be the best index to productivity in the field.

Table 1 shows the productivity of 24 second-brood females on 1- and 2-year-old camphor-tree seedlings. Six plants were examined at intervals of 1 or, less often, 2 days, beginning August 7 or 8, 1930. These dates did not necessarily represent the beginning of production for all the females on the plant, as there may have been a difference of several days in this regard. With one practically infertile exception (no. 31), there was a marked falling off in the daily production per female in the successive 10-day periods, and 50 percent of the progeny emerged in the first 6 to 13 days. At this season of the year the mean temperatures are nearly maximal. The average number of offspring per female was 110.

¹ Received for publication Nov. 5, 1934, issued April 1935. These studies cover one phase of the investigations at the laboratory formerly maintained by the Division of Fruit Insects of the Bureau of Entomology at New Orleans, La. Prior to 1926 the experiments were conducted by T. F. Catchings, and H. K. Plant was in charge of the laboratory.

² CRESSMAN, A. W., and BLISS, C. I., with the assistance of KESSELS, L. T., and DUMESTRE, J. O. BIOLOGY OF THE CAMPHOR SCALE AND A METHOD FOR PREDICTING THE TIME OF APPEARANCE OF STAGES IN THE FIELD. Jour. Agr. Research 50: 267-283, illus. 1935.

TABLE 1.—*Productivity of undisturbed second-brood females of Pseudaonidia duplex on 1- and 2-year old camphor-tree seedlings, in the insectary, 1930*

Plant no.	Date production started	Median date of production	Date production ended	Length of production period		Producing females	Total progeny	Average progeny per female	Average progeny per female per day in successive 10-day periods				
				To median date	Entire period				First	Second	Third	Fourth	Fifth
				Days	Days	Number	Number	Number	Number	Number	Number	Number	Number
30.....	Aug. 7	Aug. 18	Oct. 1	11	55	5	839	168	7.3	4.3	2.3	1.6	0.7
31.....	Aug. 8	Aug. 10	Aug. 13	2	5	1	12	12	1.0	.2			
32.....	Aug. 7	Aug. 19	Sept. 25	12	49	6	524	87	3.8	2.7	1.2	.6	.2
33.....	Aug. 8	Aug. 16	Sept. 4	8	27	2	357	178	10.4	6.0	1.2		
34.....	Aug. 7	Aug. 20	Oct. 11	13	65	4	650	162	6.8	3.9	2.4	1.9	.4
35.....	Aug. 8	Aug. 14	Oct. 9	6	62	6	266	44	2.9	4	.3		1
Mean temperature (° C.).....									27.3	26.0	25.8	25.9	23.9

In 1923, 13 first-brood females on 5 camphor-tree seedlings produced on an average 125 offspring per female and from 81 to 185 per plant. Productivity on Satsuma orange in 1924 compared favorably with that on camphor-trees, as is shown in table 2. In this case the newly emerged and settled young were removed daily or oftener.

TABLE 2.—*Productivity of undisturbed Pseudaonidia duplex females on Satsuma orange, budded stock in insectary, as determined by counts of progeny, 1924*

Brood of females	Plant no.	Date production started	Median date of production	Date production ended	Length of production period		Producing females	Total progeny	Average progeny per female
					To median date	Entire period			
					Days	Days	Number	Number	Number
First.....	1-10.....	June 15	June 23	Aug. 6	8	7	18	3,698	205
	1-11.....	June 17	June 24	Aug. 4	7	48	26	3,659	141
	1-13.....	June 15	June 26	Aug. 8	11	54	35	4,600	132
	1-16.....	do.....	June 25	Aug. 4	10	50	13	2,847	219
	1-17.....	June 16	June 30	Aug. 11	14	56	23	2,708	118
	1-18.....	do.....	June 25	Aug. 6	9	51	28	3,287	117
	1-19.....	June 17	July 4	Aug. 9	17	53	7	931	133
	Average.....				10.9	52.0			145
Second.....	2-3.....	Aug. 17	Sept. 7	Oct. 21	21	65	4	609	152
	2-5.....	Aug. 18	Aug. 28	Oct. 17	10	60	7	513	73
	Average.....				15.5	62.5			102

The possible multiplication during two generations in a single season was determined by counting the number of scales removed from each of three potted camphor-trees. By June 25, 1924, 83 scales had developed to maturity on these plants and, judged from other experiments on the same brood, about two-thirds of them may have produced offspring. As leaves dropped off from time to time, their scale populations were counted, and 8 months later, February 23-25, 1925, the number remaining on each plant was recorded. These 83 females accounted for 34,404 scales, or 415 per female. Because premature dropping of leaves through the season reduced the number of females that might normally have reproduced, this number represents a minimum rather than a typical case.

The potential progeny in an entire season in the absence of crowding, such as occurred in the preceding experiment, and without attack by parasites or predators, has been estimated from insectary experiments on 5,077 scales in 1930, covering the different parts of the life cycle. It was assumed that an average of 100 of the progeny of each female would settle successfully, and one-half of them would be females. Of these 50 females, 16.7, or 33.4 percent, would complete development and reproduce. If the same reproductive rate continued through all three generations, from 1 fertile overwintering female there would be 4,657 reproducing females the following year.

DETERMINED FROM EGGS FOUND BENEATH FIELD-COLLECTED ADULTS

Periodic counts of eggs from field-collected adults indicated that the beginning of oviposition antedated the first appearance of crawlers by from 1 to 5 weeks, depending primarily upon the time of the year and the brood. In 1926 weekly counts were made of the scale populations on camphor-tree twigs collected in Audubon Park, New Orleans. A few females laid eggs during the winter, but spring oviposition did not start until February 9, when 6 percent of the surviving females were found to be producing. Eggs had hatched beneath 5 percent of the producing females on March 16, 5 weeks later, but hatching was not general until March 30, when there were crawlers under 24 percent of the ovipositing females. Practically none of the young settled until April 6, 8 weeks after spring oviposition had started.

The long embryonic period favored the accumulation of eggs under the scale covering. From groups of individuals estimated to have started production at the same time the number of eggs increased from an average of 4.3 per female on February 9 to 64.5 (11 females, range 58 to 74) on March 23 and 81.5 (5 females, range 74 to 88) on March 30. Crawlers tended to remain under the parent scale covering for some time after hatching; under one scale there were 32 of them in addition to 48 eggs on April 6. The percentage of dead adults varied but little until May 11, when there was a marked increase. If these are assumed to be the first individuals of the overwintered females to oviposit, they laid eggs over a period of about 13 weeks, although no crawlers appeared during the first 8 weeks.

The oviposition and incubation periods during the summer were much shorter. Egg deposition by adults of the first generation in 1926 had started by June 15 in 9 percent of the females, with an average of 10 eggs each. A week later more than 60 percent had reproduced, females of comparable age showing an average of 30 eggs apiece and a few newly hatched crawlers. Many eggs had hatched, and the crawlers had emerged and settled by June 29, 2 weeks or less from the beginning of production as compared with 8 weeks in the spring. The number of eggs under each female never equaled the number found under the overwintered females, indicating that the higher summer temperature increased the rate of embryonic development more than the rate of oviposition. The first adult females of the first generation died July 20, 5 weeks after the first eggs appeared.

In 1926 both newly hatched crawlers and newly settled first-instar nymphs were found on August 17, 1 week after the first eggs were laid by second-brood females. The maximum number of eggs recorded from a single female was 63 as compared with 62 for the first-brood females and 88 for the overwintered females. The increased mortality

on September 21 showed the average reproductive period for the first individuals of the second brood to be about 6 weeks.

The span of productivity of females in the field was somewhat less than that of undisturbed females infesting potted camphor-trees (table 2). The longer interval in the insectary can probably be attributed to overlapping periods of maturity in the various females on each plant.

DETERMINED FROM OVIPOSITION IN THE LABORATORY

Productivity can be studied at closer range than by either of the two foregoing methods. When the scale coverings were removed with sufficient care from field-collected adults, it was possible to observe oviposition directly. For this purpose short sections of camphor-tree twigs bearing females were placed in Petri dishes, in the tops and bottoms of which blotting paper had been fitted, with the ends of the twigs embedded in moist sand at one edge of the dish. In this way conditions of humidity and darkness similar to those beneath the female covering were obtained, and the twigs remained green over most of the oviposition period. Freshly extruded eggs usually adhered to the posterior tip of the exposed female and were removed with a fine moistened brush at intervals of from 4 to 24 hours. The eggs removed from each female at each examination were kept separately in order that the time of hatching might be determined.

EFFECT OF TEMPERATURE ON PRODUCTIVITY

Temperature influenced the rate of oviposition more than any other factor. At constant temperatures egg laying was distributed equally throughout the 24 hours. Under fluctuating outdoor temperatures, however, fewer eggs were laid at night than during the day, especially in the spring, and in 2 out of 3 series of observations fluctuations followed changing temperatures rather closely.

In 1929 the production of 31 first-brood females kept at a constant temperature of 28° C. and of 15 first-brood females kept at 20° C. was recorded for 16 successive 6-hour intervals. The females kept at 28° laid on an average 2.42 ± 0.04 eggs in 6 hours, and those kept at 20° laid on an average 1.18 ± 0.04 eggs, as indicated in figure 1.

In 1926 second-brood females and in 1931 overwintered females were placed in Petri dishes in the thermograph shelter in the insectary, and records were kept of their production. After abnormal individuals and those that died prematurely had been eliminated, the average production per female in 12 hours was plotted against the temperature (fig. 1). The records for the second-brood adults were based on 41 females initially, but after 5 days this number was reduced to 19. The eggs were removed every 4 hours, at 1, 5, and 9 o'clock and for comparative purposes the record for each 4-hour interval was multiplied by 3. The records for the overwintered females were based on from 13 to 20 individuals and were taken at 9 a. m. and 9 p. m. A rise in temperature increased the rate of egg deposition in a fairly consistent manner, especially between 14° and 29° C. By the method of partial averages straight lines have been fitted to the points between the parallel broken lines in figure 1. Below 14° there were many cases in which production was less than expected, and in five instances it stopped altogether. Temperatures above 29° also

retarded egg laying. This optimum temperature is considerably below temperatures frequently encountered in New Orleans.

Females from the first brood of 1931 were exposed to the same range of temperature as those of the second brood of 1926, but, although the average rate of laying was nearly as high as in the earlier series, the variation from one examination to the next showed no correlation with temperature.

OTHER FACTORS AFFECTING PRODUCTIVITY

Other factors that might have affected the rate of oviposition were of minor importance in these experiments. The age of the females, as judged by the number of eggs and crawlers originally found under

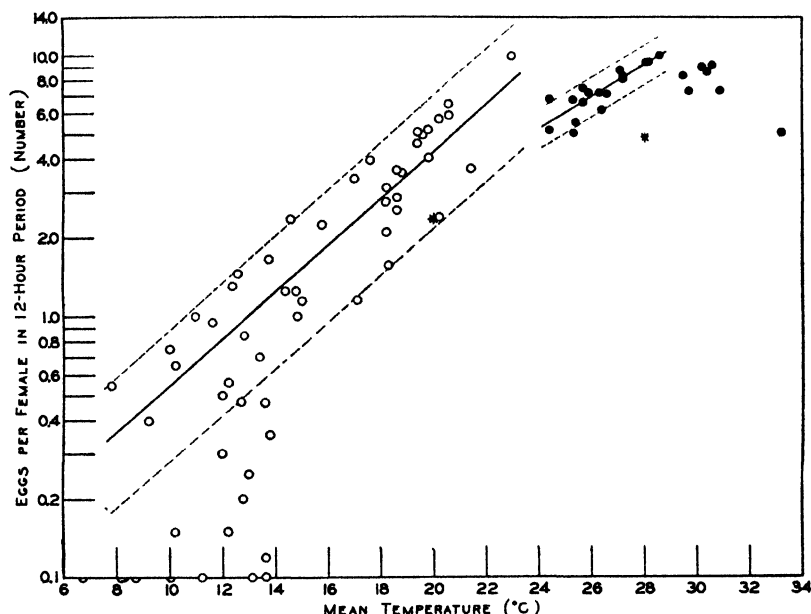


FIGURE 1.—Effect of temperature upon rate of oviposition of *Pseudonidia duplex*. Solid circles represent records of the second-brood females in 1926, empty circles represent those of the overwintered females in 1931, and asterisks (*) represent those of the second-brood females in 1929 at two constant temperatures, 20° and 28° C. The constant-temperature records have been multiplied by 2 for plotting purposes. ▤ Half circles below the base line represent zero records.

the scale covering, had no influence on the number of offspring produced during the second and third days of the experiment, when production should have been nearly normal. The manipulation necessary to remove the eggs stimulated egg laying slightly. Females kept at a constant temperature of 28° C. in August 1929 and examined at intervals of 6, 12, and 24 hours laid on an average 10.2 ± 0.2 , 9.8 ± 0.4 , and 9.0 ± 0.03 eggs, respectively, per 24 hours.

The age and size of the woody tissue on which the females had settled had a significant effect on production in the experiment started on August 24, 1926. Some of these females were on slender new growth, whereas others were on older twigs of twice the diameter. On August 27 no difference (0.04 ± 0.11 egg) was found in the average productivity over 4-hour periods, but on the following 3 days those

females on the younger wood laid on an average 1.07 ± 0.07 more eggs every 4 hours than did those on the older wood.

Females from which the scale covering had been removed would not be expected to produce a full quota of eggs, if only because of the shortened span of productivity. Nevertheless, in these experiments many of the adults laid more eggs than the average produced by the undisturbed individuals shown in table 2. Under a constant temperature of 28°C . and with examinations at intervals of from 6 to 12 hours, 22 females that had averaged 39 eggs each before the scale covering was removed laid a total of from 94 to 263 eggs, averaging 158.4 ± 4.4 , before they died or the experiment was terminated. The length of the oviposition period ranged from 9 to 19 days after the females were placed under observation. Under fluctuating temperatures 16 overwintered females just beginning production laid from 71 to 230 eggs, averaging 151, in from 38 to 50 days (average 45.6), whereas 8 females of comparable age of the next generation produced from 72 to 113 eggs, or a mean of 92.7, in from 6 to 13 days (average 9.4).

EGG STAGE

In August 1926 two experiments were conducted to obtain data on hatching and the length of the egg stage of the camphor scale. In the first experiment, each day for 8 successive days field-collected females were examined and the eggs placed on moist paper in Petri dishes, the eggs removed from each female at each examination being kept separately. The eggs from some females hatched much sooner than those from others, but all the eggs laid on a single day by each adult completed development in nearly the same time.

On the basis of similarity in the length of the developmental periods of their eggs, the 21 females isolated on August 6 could be grouped into 5 types. The mean length of the incubation period of eggs laid by each type of female on each day of the experiment is shown in figure 2. In eggs laid by females of types 1 to 3 from 6 to 8 days were required for postuterine development at the beginning of the experiment and after that the time decreased gradually. In eggs laid by females of types 4 and 5 the developmental period was comparatively brief for the first 5 days and then became slightly longer. For each type the total variation, as measured by the standard deviation, was small and about equal for eggs laid on any 1 day. Because of large differences between the means, the coefficient of variation, which usually measures inherent variability, varied enormously. For eggs laid on August 9 it ranged from 5 to more than 160 percent (table 3).

TABLE 3.—Statistical constants for length of incubation period of eggs laid by five types of *Pseudaonidia duplex* females on Aug. 9, 1926

Type of female	Mean length of egg stage	Standard deviation	Coefficient of variation	Type of female	Mean length of egg stage	Standard deviation	Coefficient of variation
	Days	Days	Percent		Days	Days	Percent
1-----	8 13	0.40	4.9	4-----	1.39	0.56	40.3
2-----	6.67	.47	7.0	5-----	.28	.45	160.7
3-----	4.95	.59	11.9				

The percentage variation in the rate of development in type 1, and possibly type 2, is about normal for homogeneous biological material—an indication that the eggs were deposited soon after they began to develop. Since the standard deviation is practically constant, differences in the length of the incubation period for eggs from the last

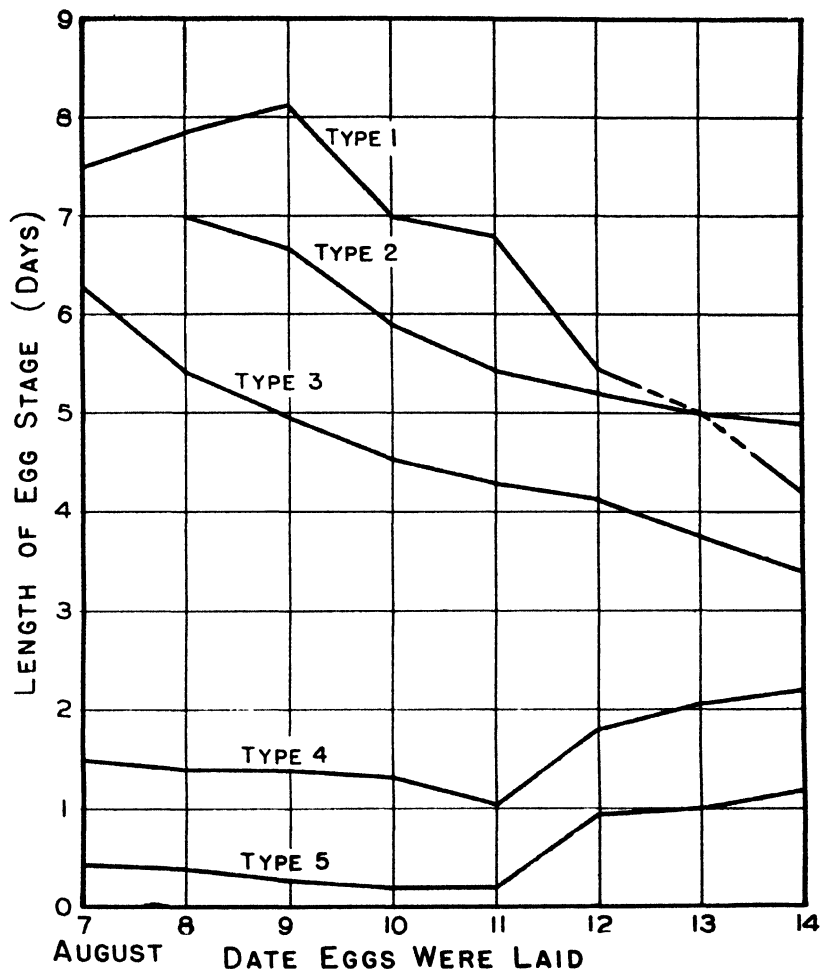


FIGURE 2 -Average length of incubation period of eggs removed from each of five types of field-collected *Pseudonidia duplex* females, August 1926

three types of females may be due to differences in the length of time they were retained in the parent's body before deposition. Diversity in age was later proved to be the most likely explanation. The two groups that laid eggs about ready to hatch were identified as the last of the first brood, while the other three groups were probably newly mature adults of the second brood, as indicated by the presence of females of the two broods in contemporary seasonal-history counts and by the rapid decrease in the length of the incubation period of the eggs of the younger females under mean daily temperatures of $28.0 \pm 0.2^\circ \text{C}$.

On August 24 the experiment was repeated, with one modification in procedure. When the scale covering was removed from each female, the number of eggs and crawlers beneath it was recorded, and also the absence or abundance of eggshells and the presence of progeny settled beneath the parent scale. The relative age of each female, as judged from these data, was then recorded in terms of the total number of eggs laid on or before August 24. Since at this time females of the second brood had been laying for about 2 weeks, individuals of all ages were available. The length of the developmental period of eggs laid subsequently was then determined as before. The 51 females used in the experiment were then divided into 6 age groups as measured by production prior to the beginning of the experiment, and for each group the average length of the egg stage was calculated for eggs laid on the first day of the experiment and for eggs laid during the first 4 days of the experiment. The results are given in table 4.

TABLE 4.—Length of egg stage of *Pseudaonidia duplex* related to production prior to the beginning of the experiment on Aug. 24, 1926

Eggs produced on or before Aug 24		Females	Mean length of egg stage for eggs laid—	
Range	Mean		On first day of experiment ¹	On first 4 days of experiment ²
<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Days</i>	<i>Days</i>
0-10	3 8 ± 0.5	17	6 86 ± 0.12	6 69 ± 0.13
11-20	17 $6\pm .8$	5	5 $84\pm .02$	4 $99\pm .43$
21-40	32 7 ± 1.5	7	4 $37\pm .15$	4 $62\pm .29$
41-60	49 2 ± 1.4	11	3 $47\pm .17$	3 $22\pm .18$
61-80	69 2 ± 2.1	5	3 $50\pm .21$	2 $88\pm .11$
81-100	86 8 ± 1.4	6	2 $67\pm .22$	1 $96\pm .21$

¹ Averages for individual eggs.

² Averages of means for each female.

There was a close correlation between the production age of the female and the length of time fertilized eggs were held before being deposited. This was demonstrated both by the shortening of the egg stage as the female aged and by the fact that, for females of the same age group, the average for the first 4 days was in every case less than that for the first day only. After eggs had begun to hatch and crawlers to emerge, the previous production of a female could be determined only approximately, an uncertainty that applied particularly to the oldest group. Oviposition probably started soon after the first eggs began developing, as judged from the 10-percent coefficient of variation in the length of the egg stage for eggs laid by the youngest group of females.

In 1931 eggs were removed from overwintered females and females of the first brood that were just beginning to reproduce. As in previous experiments, the females could be grouped into a small number of classes on the basis of similarity in the length of the egg stage. Observations on overwintered females were started February 25, with egg removals at 12-hour intervals. The average length of the incubation period of eggs from 9 representative females has been plotted in figure 3, together with that of eggs from 2 females that varied most from the average. Since the temperatures increased

from the beginning to the end of the experiment, the shortening of the egg stage was due to a rising temperature as well as to an increase in the degree of embryonic development prior to oviposition.

Observations on the first-brood females were begun on June 24, with egg removals at 4-hour intervals. Although of nearly the same production age, these females showed a variation of nearly 2 days in the length of time eggs were retained before being deposited. As in the earlier experiments, the later eggs hatched in a much shorter time than the first ones to be laid.

Most of the eggs laid on a single day by a given female hatched within a short period, but there were some exceptions. Occasionally

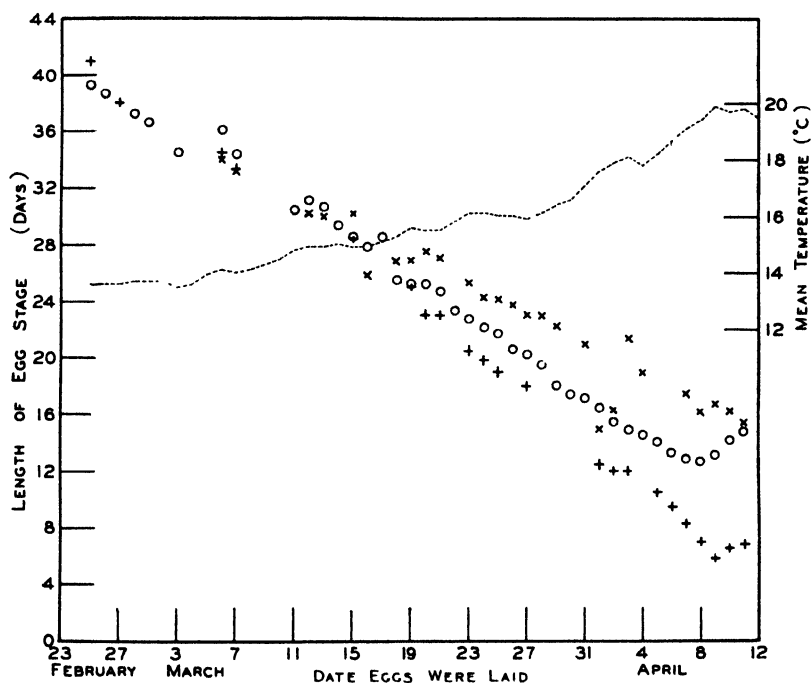


FIGURE 3. - Average length of incubation period of eggs of *Pseudonidia duplex* laid by overwintered females in 1931. The circles represent averages of eggs from 9 typical females, the two series of crosses (+ and X), eggs from the 2 females, in a total of 23, which varied most widely. The temperatures, represented by the dotted line, are the means for the incubation period of typical eggs hatching at each observation.

eggs adhered in strings as they were laid. In removing these care was taken to preserve the order of laying, so that it might be compared with the order of hatching. In general, the eggs hatched in the order in which they were laid, but some eggs hatched from 12 to 24 hours before older ones in the string. In 1931 the incubation period of a few eggs differed by 2 or 3 days from that of the majority of the eggs laid on the same day. Some of these exceptional eggs had apparently just begun to develop when deposited; others were laid in such an advanced stage that they hatched during the 4-hour interval between egg removals.

Because of the influence of the age of the parent, only the first eggs to be laid after removal of the coverings were deposited sufficiently early to permit measuring the effect of temperature upon the rate of

embryonic development. To determine this factor, observations were made on the length of the developmental period of such eggs taken from females of each brood, thus representing three different seasons of the year. The results have been summarized in table 5. When the mean temperature of the incubation period was 13.6° C., an average time of 39.6 days was required to complete development, whereas the eggs hatched in 6.8 days under a mean temperature of 27.1° C.

TABLE 5.—Effect of temperature on the length of the developmental period of eggs of *Pseudaonidia duplex* laid on the first day of observation by females that had laid previously an average of 3.6 eggs

Date eggs were laid	Brood of females	Females	Eggs	Mean temperature during incubation	Length of egg stage			
					Mean	Minimum	Maximum	Coefficient of variation
		Number	Number	° C.	Days	Days	Days	Percent
Aug. 25, 1926	Second	17	70	26.9	6.86±0.12	5	8	10.3
Feb. 25, 1931	Overwintered	8	8	13.6	39.62±.18	39	41	1.8
June 24, 1931	First	11	25	27.1	6.82±.04	6.2	7.5	4.7

The mortality of eggs removed to Petri dishes ranged from 10 to 20 percent in warm weather. Under the low temperatures of early spring many more were killed by fungi, a thing that did not occur in the field. Eggs placed in open Petri dishes and kept in closed incubators with fan circulation failed to hatch, but when fresh air was introduced continuously by a filter pump, hatching compared favorably with that in the insectary. Mortality in the field could be estimated approximately from the number of dead eggs and crawlers under spent females. In the first and second broods the mortality was probably less than 5 percent of the total production, but in the third brood the mortality of eggs, and especially that of crawlers, was much greater.

EMERGENCE AND SETTLING OF THE CRAWLER

EMERGENCE

The newly hatched crawler did not leave the protection of the parent scale immediately, but was responsive to external conditions. This was indicated particularly by the diurnal variation in the number emerging. In August 1923 potted camphor-tree plants with undisturbed reproducing females were examined at 6 and 9 a. m., 12 m., and 6 p. m. for several days, at which times all crawlers and newly settled first-instar nymphs were removed. Crawlers seldom emerged during the night, although a few newly settled individuals with white covers were found at 6 a. m. It is probable that these had appeared the preceding day but had been overlooked, as in a study of night emergence during the summer of 1925 no crawlers were found to emerge during the night even when the plants were illuminated with electric lights. From 6 a. m. to 6 p. m. the rate of emergence varied considerably, as is indicated in table 6. Usually more than half the crawlers left the parent scale covering between 9 a. m. and noon, although the proportion varied from day to day.

TABLE 6. — Number of crawlers of *Pseudaonidia duplex* emerging from beneath undisturbed females on potted camphor-trees in different intervals during the day, August 1923

Females	Plants	Date	Mean temperature 9 a. m. - 12 m.	Crawlers emerging in interval—				Total 6 a. m. to 6 p. m.
				6-9 a. m.	9 a. m.-12 m.	12 m.-3 p. m.	3-6 p. m.	
Number	Number		° C.	Number	Number	Number	Number	Number
47	6	Aug. 14.....	28.9	5	63	24	13	105
		Aug. 15.....	28.9	24	38	20	11	93
		Aug. 16.....	29.1	6	46	5	12	69
		Aug. 17.....	30.2	10	21	10	6	47
		Aug. 18.....	29.6	1	28	14	2	45
		Average.....		9.2	39.2	14.6	8.8	71.8
55	4	Aug. 21.....	30.4	24	42	9	9	84
		Aug. 22.....	29.5	12	52	11	11	86
		Aug. 23.....	29.4	1	35	1	13	50
		Aug. 24.....	28.7	4	56	10	5	75
		Aug. 25.....	28.8	17	37	3	5	62
		Aug. 26.....	29.4	16	21	6	3	46
		Average.....		12.3	40.5	6.7	7.7	67.2

The time of emergence depended in part upon the temperature. The warmer the day the earlier the crawlers tended to emerge. When the mean temperature between 9 a. m. and 12 m. was 25° C., 80 percent of the daily emergence occurred during that time, but when the mean temperature for this interval increased to 31° C., enough crawlers emerged before 9 a. m. to reduce the emergence between 9 a. m. and 12 m. to 40 percent of the daily total.

The effect of temperature upon the percentage of crawlers emerging between 11 a. m. and 2 p. m. each day in August 1923 and in March 1925 is shown in figure 4. During the summer, when the minimum temperatures were high, the total number of crawlers that emerged on any day depended largely upon the number that hatched on that or the preceding day, and not on the temperature. For August, therefore, the emergence between 11 a. m. and 2 p. m. has been calculated as a percentage of the number that emerged for the entire day. In the spring, however, temperatures were too low for crawlers to come out from the covering, as was shown by the greatly reduced emergence on cool days as compared with that on warm days. In March the daily emergence of crawlers from 39 mature females on 6 plants depended so largely upon temperature that emergence between 11 a. m. and 2 p. m. could not be referred satisfactorily to the emergence for the entire day, as in the summer. Instead, the daily totals were plotted against the date, and a smooth curve (not shown) was fitted to the records for those days when low temperatures did not retard emergence. This curve was principally a correction for the continuously decreasing production that has been noted previously in the paper. The number of crawlers emerging between 11 a. m. and 2 p. m. has been converted into a percentage of the number expected from the curve, and in figure 4 this percentage has been plotted against the average temperature of the interval.

It is shown that when midday temperatures fell below 20° C. very few crawlers emerged, either then or at any other time during the day. With midday temperatures of 23° C. and above, it is probable that temperature did not retard emergence.

External conditions that brought crawlers out onto the plant favored crawling. Of the individuals reported in table 6, few that emerged in the morning were settled at the time of examination, but

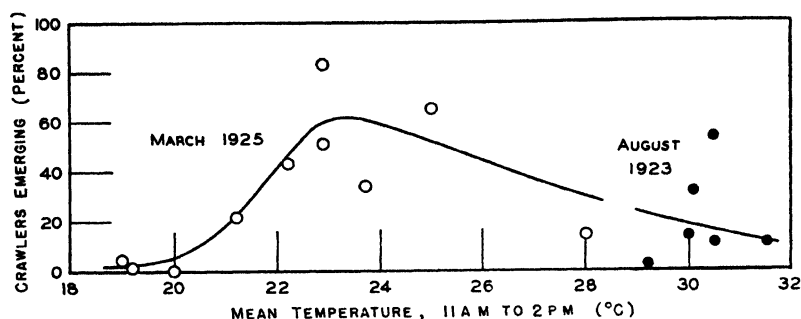


FIGURE 4.—Percentage of emergence of crawlers of *Pseudaonidia duplex* at midday (11 a. m. to 2 p. m.) as related to the temperature at this time, March 1925 (empty circles) and August 1923 (solid circles). The percentage emerging in March is based upon an estimated possible daily total, emergence in August is a percentage of the observed daily total.

late in the afternoon a fair proportion had settled in the 3-hour interval.

After emerging, the nymph generally crawled toward the light, a tendency that kept the population moving out onto the younger growth of the plant. During the winter of 1927 counts were made of

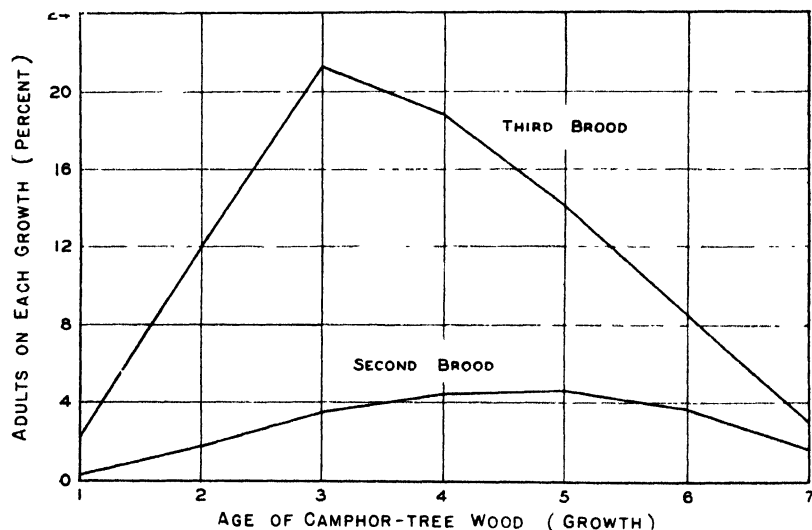


FIGURE 5.—Percentage distribution of adult females of the second and third broods on camphor-tree wood of different ages in the winter of 1927, showing the movement of the new brood toward the younger growth.

scales on camphor-tree wood of different ages, the counts of old spent females of the second brood being kept separately from those of overwintering females of the third brood that had not yet produced. The percentage occurrence of each type of female on wood of each growth is plotted in figure 5. This shows that the scales of the new generation, comprising 80 percent of the total, had moved

about one growth toward the newer wood. As there are normally three growths of camphor-tree wood and three generations of the camphor scale each year, this rate of migration should keep the population on the same growth throughout the year.

RATE OF CRAWLING

Because of its importance in the spread of the camphor scale, crawling was studied experimentally. Adult scales were scraped from infested twigs onto sheets of paper. Active uninjured nymphs crawled free of the debris toward the light and could then be transferred on the tip of a fine moistened brush to a potted plant.

An apparatus was set up in the constant-temperature room for investigating the accuracy of orientation and the rate of crawling in relation to light intensity and temperature. This apparatus consisted essentially of a sliding wooden stage on which a sheet of smooth, black, unglazed paper measuring $5\frac{1}{2}$ by 7 inches was fastened, and a light source, usually a 75-watt mazda bulb, which supplied unilateral light filtered through a copper chloride solution at a level slightly above that of the stage. A crawler was placed on the sliding stage as far as possible from the light source, and the stage was moved back as fast as the insect crawled toward the light, so that it was illuminated uniformly throughout its trail. As it crawled, the larva was followed closely with a white pencil and its positions at 30-second intervals were indicated by cross marks. A mercury thermometer was suspended over the crawler and read at the beginning and end of each 5-minute trail. There was sometimes a difference as great as 1.3°C . between the temperature at the beginning and the end of a trail, because of the differential on which the thermostat operated. In such cases the temperature was interpolated for any given section of the trail.

To obtain as consistent a group of records as possible, the distance crawled in 1 minute without marked change in the angle of orientation was adopted as the unit, and as many 1-minute records as possible were selected from the many original records. Some crawlers gave five or more unit records; for others none were available. For each unit the angle of orientation from the light source, the most probable temperature (by interpolation), and the distance crawled were determined. The distance was measured by enlarging the trail five times with a pantograph, measuring the enlarged trail with a map measure, and converting back to centimeters of original trail.

The three typical trails reproduced in figure 6 illustrate the irregular orientation that was encountered, as well as the greater speed of crawling at the higher temperatures.

These experiments were started in June 1929 with second-brood crawlers and completed in September with third-brood crawlers. It was found that the crawlers did not behave uniformly. The majority of trails at a given temperature checked closely with one another, but a few nymphs crawled consistently much more slowly. These were designated as "slow" individuals and handled separately from the others. They crawled about one-half as fast and oriented themselves less accurately toward the light. They were more frequent toward the end of production by the second brood in September (20 percent of the total) than in June, perhaps because of the lower

vitality of crawlers late in the season. Some of them, however, may have been sluggish because they were isolated too soon after hatching, a condition that would not occur in the field. They will not be considered further.

The relation of temperature and light intensity to rate of crawling was tested over a temperature range of from 17° C. to 37° C. under a 75-watt lamp, a 15-watt lamp, and in early morning sunlight. A 75-watt light delivered to the crawlers a radiation intensity of 0.0029 to 0.0038 gram-calorie per square centimeter per minute. It was found that the observations at 75 watts could be adequately fitted with a straight line by the method of partial means (fig. 7), indicating that the percentage increase in rate of crawling per degree rise in temperature was constant over the entire range of temperature. This is in contrast to other activities of the camphor scale, such as oviposition and settling, which were frequently retarded by temperatures above 30° C. A reduction of 80 percent in light intensity (to approximately 0.007 gram-calorie per square centimeter per minute) by substituting a 15-watt bulb did not change the rate of crawling; therefore, these observations have been used with those under 75-watt illumination in determining the curve. The greater intensity of early morning sunlight, however, increased the rate of crawling materially.

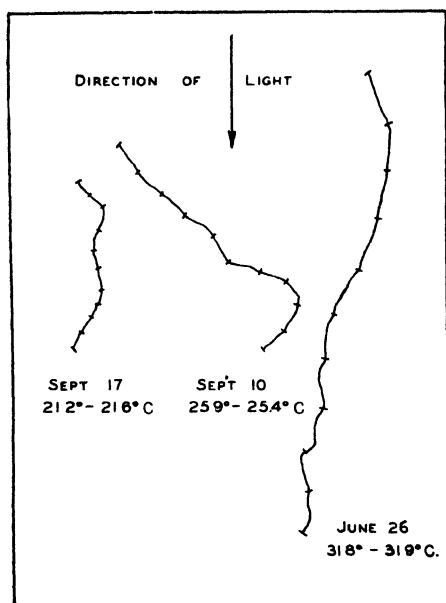


FIGURE 6.—Specimen trails of *Pseudonidia duplex* crawlers under unilateral illumination of 75-watt bulb, showing type of orientation in the trail of a single crawler and the relative speed of crawling at different temperatures. The cross marks were made at 30-second intervals. One-fourth natural size.

in the course of a 5-minute trail the crawler sometimes shifted both the angle and the general direction of orientation. Several crawlers described complete circles under unilateral illuminations. The angular departure from the direction of the light rays was greater at the lower than at the higher temperatures, averaging from 36° to 54° at 17° C. to 19° C. and from 13° to 18° at 33° C. to 36° C. A closer correlation between the angle of orientation and the rate of crawling is shown in figure 8, together with the number of 1-minute trails for each 5° of angular departure from the direction of light. It is shown that few nymphs crawled directly toward the light. The average angle of departure from precise orientation was 28.1°. As the orientation became less exact, the rate of crawling diminished by a constant percentage per degree of angular departure (upper curve in fig. 8).

Within the range of light intensities used in these experiments, an increase in light decreased the accuracy of orientation if it had any effect at all. The average angular departure from direct orientation toward the light was $23.1^\circ \pm 1.3^\circ$ at 15-watt illumination, $28.1^\circ \pm 0.6^\circ$ at 75-watt illumination, and $33.9^\circ \pm 2.2^\circ$ in early morning sunlight.

RATE OF SETTLING

The crawler is more exposed to death while it is active than after it has attached itself to the host by settling. Accordingly, the rate at which settling occurred has been studied in some detail. The ends of

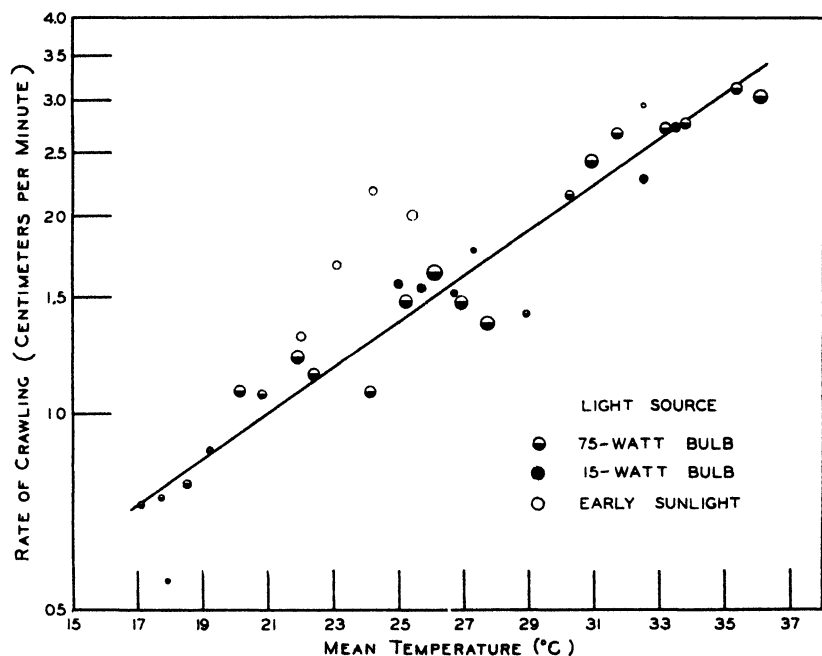


FIGURE 7.—Relation between rate of crawling of *Pseudaonidia duplex* nymphs and temperature under light intensities of a 75-watt lamp, a 15-watt lamp, and early morning sunlight, in a constant-temperature room, 1929. A logarithmic curve has been fitted to the 75-watt and 15-watt data. The diameter of each circle is proportional to the square root of the number of 1-minute trails upon which the reading is based.

short sections of second- or third-growth camphor-tree twigs, each with a single leaf, were sealed in shell vials containing water, and each twig was mapped and then infested with 20 crawlers. The position of each inactive crawler was noted hourly, and the time of settling was based upon the first observation at which the crawler was found in its final location. A few twigs (5 out of 25) on which the settling was abnormally low, presumably as a result of handling, were discarded. Two series of observations were carried out in 1926 on third-brood nymphs at midday before a large east window. The experiment was repeated on the first-brood crawlers in the spring of 1929 under artificial illumination from two 75-watt lamps in the constant-temperature room. The records are summarized in figure 9 and table 7.

TABLE 7.—Rate of settling of *Pseudaonidia duplex* crawlers after being transferred to camphor-tree twigs in the laboratory

Date	Temperature		Kind of light	Twigs	Crawlers	Total crawlers settling		Survivors settling —		
	Mean	Range						In 3 hours	In 6 hours	On stem
1926	°C.	°C.		Number	Number	Number	Percent	Percent	Percent	Percent
Sept. 23.....	30.2	1.9	Daylight.....	10	200	126	63.0	78.8	92.9	72.2
Sept. 28.....	32.2	2.5	do.....	10	200	86	43.0	89.4	98.8	37.2
1929										
Mar. 27.....	28.0	1.0	Two 75-watt lamps.	11	220	168	76.4	86.9	98.2	37.5
Mar. 28.....	27.5	1.0	do.....	12	240	163	67.9	78.5	90.2	46.3
Apr. 1.....	22.5	1.0	do.....	12	240	100	41.7	72.0	91.0	57.0
Apr. 2.....	22.5	1.0	do.....	12	240	92	38.3	70.7	94.6	52.2

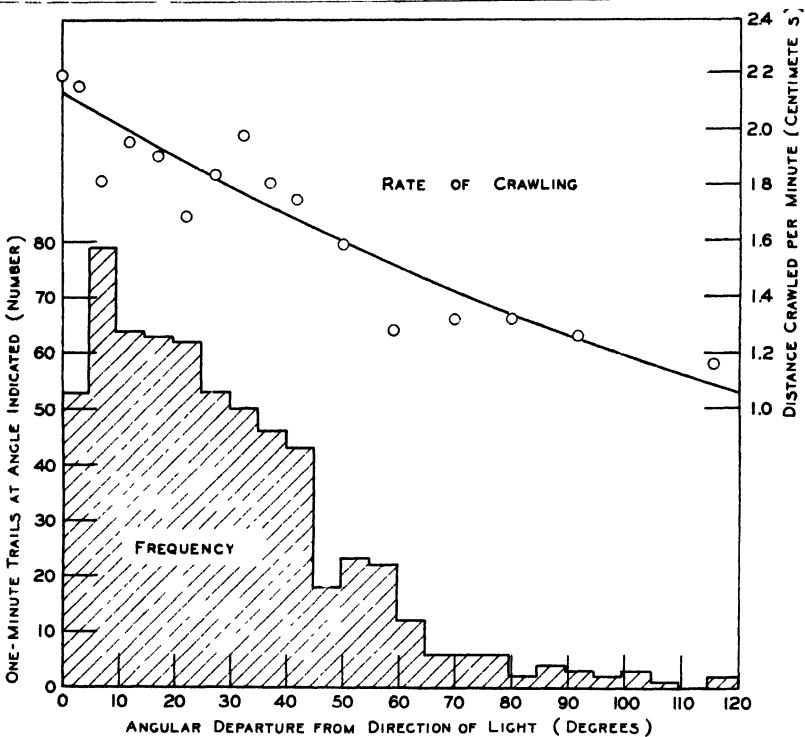


FIGURE 8.—Accuracy of orientation of *Pseudaonidia duplex* in crawling toward light from a 75-watt bulb as shown by the frequencies of angular departure and the rate of crawling at different angles.

In 1929 nearly twice as many crawlers settled at 27.5° C. as at 22.5° C., and those that did settle successfully did so more rapidly at the higher temperature. In the higher temperature range of the two experiments in daylight in 1926 fewer settled than at 27.5°, and though only two-thirds as many survived at 32.2° C. as at 30.2° C., the survivors settled more rapidly. Temperature changes in either direction from an optimum of 27° to 30° C. apparently reduced the number of survivors. Within the entire temperature range of 22° to 32° C., however, more than 90 percent of the crawlers

that survived settled in the first 6 hours after being transferred to the twigs.

Most of the males are found on the leaves and most of the females on the wood of the camphor-tree. The rates of settling in the two positions have been compared in order to determine any possible relationship to sex differences in the crawlers. At the end of 2 hours at 27.5° C. 46 percent had settled, divided about equally between the stem and the leaves and petioles. Of those that required more than 2 hours to settle, 71 percent were found on leaves and petioles. Although this seemed to indicate that crawlers on leaves, mainly

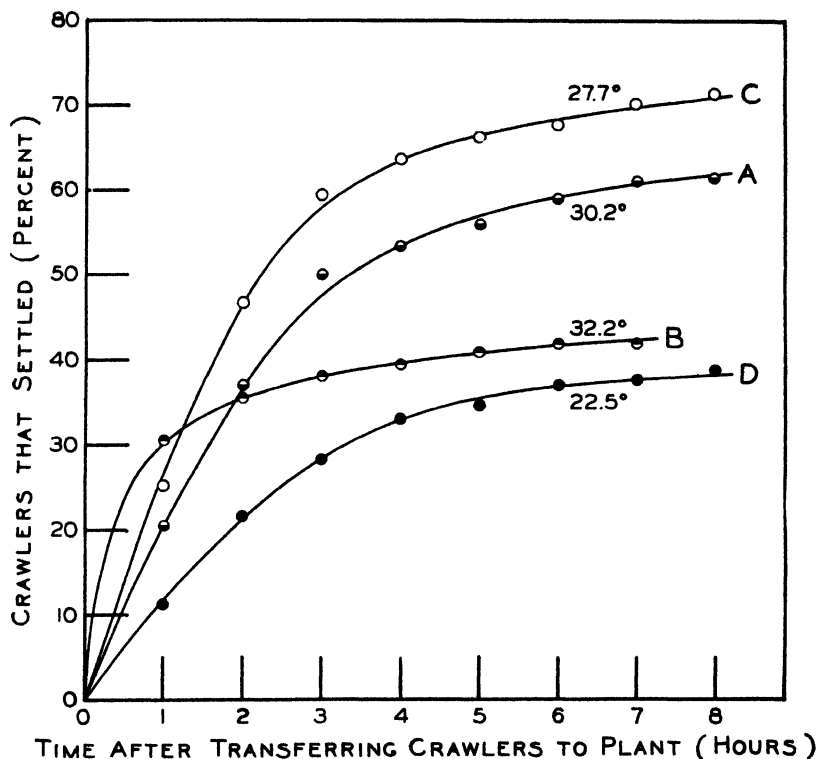


FIGURE 9. Rate of settling of *Pseudanidia duplex* crawlers on camphor-tree twigs and leaves: A and B, in daylight at 30.2° and 32.2° C., respectively, September 1926. C and D, under illumination of two 75-watt lamps, at 27.7° (mean for Mar. 27 and 28) and 22.5° (mean for Apr. 1 and 2), respectively, 1929.

males, required slightly longer to settle than those on stems, principally females, a comparison of the average time in the two positions showed no significant difference, being 2.37 ± 0.11 hours on the stem and 2.50 ± 0.08 hours on the leaves and petioles.

The positions of settling in the foregoing experiments have been classified in table 8. Of those on the stem, from 78 to 98 percent settled about nodes, bases of petioles, or similar irregular places. On the upper and lower surfaces of the leaves 86 to 100 percent settled next to the midrib and most of the remainder along large veins. The different parts of the leaf were favored as follows: 67 percent on the upper surface, 15 percent on the lower surface, and 18 percent on the petiole.

TABLE 8.—*Positions in which crawlers of Pseudaonidia duplex settled on small twigs, each with a single leaf, in the laboratory, 1926 and 1929*

Part of twig	Number of crawlers settling on date indicated							Total
	1926 ¹		1929 ²					
	Sept. 23	Sept 28	Mar 26	Mar 27	Mar. 28	Mar. 30	Apr. 1	
Stem								
About nodes, etc.-----	89	29	68	55	63	46	50	400
Between nodes.-----	2	3	19	10	8	5	7	5
Leaf:								
Upper surface-----								
Midrib-----	30	48	50	73	48	41	20	310
Blade-----	0	0	10	7	6	3	1	2
Lower surface-----								
Midrib-----	0	2	24	10	11	7	10	6
Blade-----	0	0	2	2	0	3	2	9
Petiole								
Top-----	5	3	9	13	10	5	5	54
Bottom-----	1	0	1	9	15	9	5	46

¹ In daylight.² In constant-temperature room under artificial light, except on Mar. 26, when the observations were made in daylight.

FACTORS LIMITING THE NUMBER SETTLING

Many experiments were conducted in which crawlers were obtained from field-collected females, by methods already described, transferred by brush to potted plants, and the number that settled counted a day or two later. This method was subject to error from many causes, such as the premature transfer of crawlers, injury in handling, and abnormally adverse conditions on individual plants, peculiar to experimentation. In certain earlier experiments plants were omitted which seemed to fall in the last category, as judged by poor settling in comparison with the majority in the same group. Even with nearly ideal experimental conditions, the crawlers were affected by so many factors that the number that survived was unpredictable. As the experiments progressed, some of the more important conditions that reduced settling were disclosed, and this information was used wherever possible to improve the technic of the later experiments.

TEMPERATURE

The effect of temperature has already been shown in the experiments on rate of settling. The influence of temperature was, however, frequently masked by other factors. A series of experiments was therefore conducted under controlled conditions in which the other factors were kept as nearly constant as possible. On September 5, 1929, between 7:30 a. m. and 12:30 p. m., 100 crawlers from the same source were transferred to each of 30 plants and the plants were then placed in five chambers, each at a different temperature. The number that settled and formed complete covers at each temperature is shown in table 9 and figure 10. More crawlers settled at the higher temperatures, but the effect was not uniform with all the individuals. Apparently those settling on leaves were favored more by a rise in temperature than those on the stem. This tendency was observed also in the last column of table 7.

TABLE 9.—Settling of crawlers of *Pseudaonidia duplex* on potted camphor-tree plants at different constant temperatures, morning of Sept. 5, 1929¹

Temperature (° C.)	Crawlers settled	First-instar nymphs on stem	Temperature (° C.)	Crawlers settled	First-instar nymphs on stem
	Percent	Percent		Percent	Percent
15.2.....	24.5±2.0	59.6±6.8	25.7.....	59.5±2.8	51.3±2.0
19.4.....	46.2±2.3	56.9±1.3	29.2.....	67.3±2.7	45.6±2.4
22.1.....	64.7±2.2	55.6±2.9			

¹ 100 crawlers on each of 6 plants at each temperature.

Settling records taken under outdoor temperatures in the shaded insectary or in the unheated laboratory also showed the effect of temperature. As these records were subject to variation in crawler

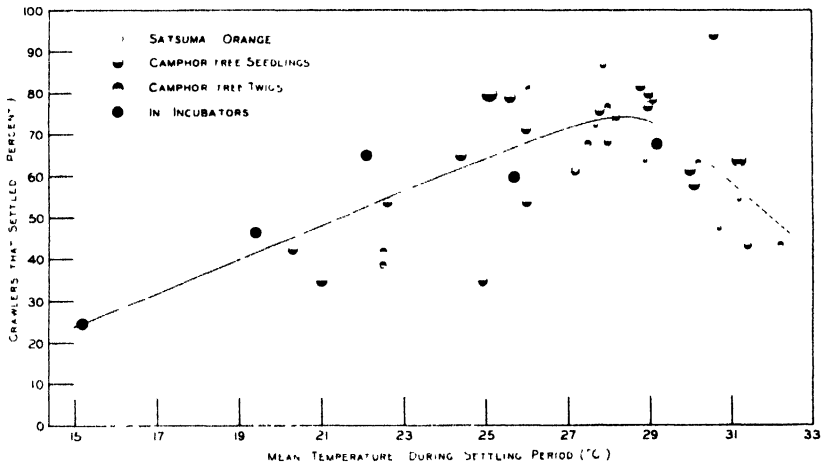


FIGURE 10.—Effect of temperature upon percentage of crawlers settling on different types of material. Original data in tables 7, 9, and 10. The diameters of the circles are proportional to the square root of the number of plants included in each mean.

material on different days, only crawlers transferred on a single day or half day were included in any given average, a procedure which increased both the validity of each mean and the variation between the means. The data have been summarized in table 10. The temperature was the average for the 6 hours following infestation when the exact time was recorded, an average for the period from 10 a. m. to 4 p. m. in the case of plants known to have been infested some time in the morning (1930), and an average from 10 a. m. to 8 p. m. when only the date was recorded. Most of the temperature records were taken from the insectary thermograph, and usually were sufficiently close to the temperatures of the plants, though occasionally, during rapid changes in outdoor temperature, there may have been a lag due to the absence of air currents about the plants. Beginning in 1926, plants were carefully protected from air currents during and after infestation, a procedure which undoubtedly increased the uniformity of settling.

TABLE 10.—Settling of crawlers of *Pseudaonidia duplex* transferred to camphor-tree and Satsuma orange seedlings in shaded insectary or unheated laboratory

CAMPHOR-TREE

Date	Time of day	Mean temperature	Plants	Total crawlers transferred	Crawlers settled	
		°C	Number	Number	Number	Percent
1924:						
Apr. 8	p. m.	22.6	4	800	427	53.4
June 18		31.2	1	100	54	54.0
June 20		31.4	3	600	257	42.8
July 3		27.2	4	800	487	60.9
July 31		30.7	1	100	47	47.0
Aug. 18	p. m.	27.7	1	200	144	72.0
Aug. 19	a. m.	28.9	1	200	127	63.5
Do	p. m.	28.2	3	600	422	70.3
Aug. 20	a. m.	28.0	3	600	407	67.8
1925, Apr. 21		27.9	2	400	346	86.5
1926:						
Sept. 9	a. m.	31.2	11	900	571	63.4
Do	p. m.	30.1	7	600	344	57.3
Sept. 16	a. m.	29.1	3	450	351	78.0
Do	p. m.	27.8	4	600	452	75.3
1928						
Aug. 30		26 ±	5	750	532	70.9
Sept. 8		29 ±	5	750	574	76.5
Sept. 14		29 ±	5	750	597	79.6
Sept. 18		26 ±	4	600	321	53.5
1929, Sept. 4	a. m.	30 ±	6	600	366	61.0
1930:						
Mar. 7	a. m.	20.3	5	1,500	629	41.9
Mar. 18	a. m.	24.9	6	1,800	622	34.6
Mar. 27	a. m.	21 ±	6	1,800	622	34.6
Apr. 30	a. m.	24.4	6	900	582	64.7
June 17	a. m.	30.6	5	750	702	93.6
Sept. 2	a. m.	28.8	4	600	488	81.3
Sept. 15		25.1	12	1,800	1,432	79.6
Oct. 2	a. m.	25.6	6	900	709	78.8

SATSUMA ORANGE

1924							
Apr. 8	a. m.	23.8	7		1,400	1,026	73.3
Do	p. m.	23.2	7		1,400	975	69.6
June 18		31.2	3		600	371	61.8
June 19	p. m.	27.2	3		600	368	61.3
June 20		31.4	8		1,600	1,211	75.7
June 24		29.6	2		400	238	59.5
July 3		27.2	1		200	138	69.0
July 31		30.7	2		200	115	57.5
Aug. 18	p. m.	28.0	3		600	314	52.3
Aug. 19	p. m.	28.2	3		600	438	73.0
Aug. 20	a. m.	28.1	8		1,600	966	60.4

¹ Unequal numbers on different plants equalized on basis of 100

The relation between temperature and the percentage of crawlers that settled and formed covers, under controlled conditions on camphor-trees, and under sheltered outdoor temperatures on camphor-tree and Satsuma orange plants, has been plotted in figure 10. In the temperature range from 15° to 29° C. each degree centigrade increase in temperature tended to increase the number settling by about 3.5 to 4 percent, representing an average survival range of from 25 to 75 percent, but above this temperature there was usually a reduction in the number that settled. It has already been noted that during cool weather crawlers sometimes remained under the parent scale covering and delayed emergence until the midday temperature reached 20° C. If this occurs generally, settling in the field would seldom average below 40 percent of normal emergence. On the other hand, on very warm days the earlier morning emergence of crawlers might also increase survival.

AIR CURRENTS

Although most of the emerged crawlers were easily dislodged by the wind, more survived than had been anticipated. On September 16, 150 crawlers were placed on each of 13 plants in still air, and immediately every alternate plant was placed 24 inches from a 12-inch oscillating fan. These plants were thus exposed intermittently to a maximum air velocity of 12.7 miles per hour, or an average of 5.3 miles per hour, at temperatures from 28° to 30° C. On the other 7 plants, which remained in still air, 76.4 ± 1.3 percent survived, but on the 6 plants under the fan only 37.4 ± 0.9 percent survived. Of these, less than half could have inserted their beaks in the 20 to 35 minutes required to infest the plants in still air before they were placed before the fan. Hence, one-fourth to one-third of the active crawlers were able to settle in a fairly strong intermittent breeze. In the field, however, some of the crawlers dislodged by the wind were probably carried to other host plants.

Wind velocities equal to those under the fan were estimated (from Weather Bureau data) to occur, in the natural habitat of the camphor scale in New Orleans, on 1 day in 5 in April, but only on 1 day in 30 in July and August.

LIGHT

The number of crawlers settling in the light or in moderate shade has been compared with the number settling in an adjacent paper dark box. On September 16, 1926, 150 crawlers were transferred to each of 13 potted camphor-trees, 7 of which were placed in the dark box immediately after being infested. A subsequent count showed that 76.4 ± 1.3 percent settled in the light, and only 64.8 ± 2.8 percent settled in the dark. The difference, 11.6 ± 3.1 percent, was significant.

The difference between males and females in the position of settling was somewhat less pronounced upon the camphor-trees in the dark box than upon adjacent plants in daylight. The percentage distributions of each sex upon leaf blades, leaf petioles, and stems in the light and in the dark are compared in table 11 for scales which developed to a stage in which the sexes could be separated. The experiment of 1926 was repeated in 1929 with this modification: Field infestations were scraped into small cones on the stem from which crawlers emerged and infested the plant over a period of 3 days, on some plants in daylight, on others in the dark. The duplicate plants for each treatment and sex were compared with one another by means of the χ^2 test to determine whether the proportions of crawlers which settled upon any given part of the plant were equal within the limits of chance. In both seasons parallel plants differed more from one another in two or more categories than could be attributed to chance, so that the significance of the different comparisons has been determined from the observed variability by the analysis of variance, as described by Fisher.³

³ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, pp. 178-237. Edinburgh and London, 1928.

TABLE 11.—Comparison of position of settling of sexes of *Pseudaonidia duplex* in reference to light

[In 1926 the crawlers were transferred by brush on Sept. 16, and the scales counted on Oct. 18–Nov. 15. In 1929 scale material was scratched from field infestations, and placed in a small cone on the stem from which crawlers emerged and infested the plants Sept. 2–6, scales were counted Oct. 23–24]

Year and sex	Condition	Plants	Crawlers that settled on —				Variance in 3 positions of settling	Degrees of freedom	Sum of squares	Mean square	L_n of S. D.	z
			Scales	Leaf blades	Leaf petioles	Stem						
		Number	Number	Percent	Percent	Percent						
1926.	Male....	(Light.. 6	132	47.7	32.6	19.7	Light v. dark	2	204.96	102.48	2.31	0.68
		(Dark.. 7	191	37.2	36.7	26.2	Experimental error....	22	580.43	26.38	1.64	
	Female....	(Light.. 6	150	2.7	4.1	92.6	Light v. dark	2	324.12	162.06	2.54	1.87
		(Dark.. 7	141	14.9	5.0	80.1	Experimental error....	22	83.64	3.80	.67	
1929.	Male....	(Light.. 7	791	86.8	10.7	2.5	Light v. dark	2	256.48	128.24	2.43	1.17
		(Dark.. 5	219	82.2	10.5	7.3	Experimental error....	20	249.62	12.48	1.26	
	Female....	(Light.. 7	916	21.0	3.2	75.8	Light v. dark	2	1055.08	527.54	3.13	.60
		(Dark.. 5	302	26.8	5.3	67.9	Experimental error....	20	3180.76	159.04	2.53	

The results show that of the males, which normally occur sparingly on the stem or wood of camphor-trees, a larger percentage settled on the stem in the dark than in the light in both experimental series. Of females, more settled on the leaves in the dark than in the light. This difference was not vitiated by the relatively heavier infestations of both sexes on the stems of the smaller plants used in 1926 than occurred on the larger plants in 1929. Despite the relatively large variability between plants, the absence of light modified the position of settling significantly. The chance that the distribution on leaf blades, leaf petioles, and stems in the dark differed from that in the light exceeded 100 to 1 for females in 1926 and males in 1929, and nearly equalled or exceeded 20 to 1 for the other two categories. Most of the variability between parallel plants in 1929 was due to two plants, one in the dark and the other in the light, both showing an abnormally large amount of settling on the stem. If these two records are omitted, the duplicate tests are more nearly in agreement (by the χ^2 test), and the effect of light is statistically significant for both sexes ($n_1=2$, $n_2=16$; male $z=0.77$, female $z=0.92$). From this we may conclude that a small part of the differential settling of the two sexes is clearly a visual response. An alternative explanation by differential mortality proved inapplicable.

SPECIES OF HOST

In 1924 the settling of crawlers on Satsuma orange was compared with that on camphor-trees. Table 10 shows 8 days or half-days when crawlers were placed on both camphor-tree and Satsuma orange plants. More crawlers survived on Satsuma orange than on camphor-trees in 6 of these cases, or an average of 6.4 percent more for the 8 cases. This difference was not statistically significant, indicating that Satsuma orange was no more susceptible to infestation than the camphor-tree. Many more female scales were found on Satsuma orange leaves than on camphor-tree leaves.

TIME OF TRANSFERENCE

Crawlers normally emerged from under the parent scale in the morning, but they could be obtained readily in the afternoon for infesting plants. On several days plants were infested both in the morning and in the afternoon with crawlers freshly scratched from the same field-infested material. (Table 12.) In 6 out of 7 cases

TABLE 12.—Comparison of settling of crawlers of *Pseudaulnidia duplex* in the morning and in the afternoon of the same day

Date	Total crawlers transferred		Crawlers settled	
	In the morning	In the afternoon	In the morning	In the afternoon
	Number	Number	Percent	Percent
1923				
June 11	1, 173	750	38. 6	34. 1
June 12	1, 250	1, 500	42. 6	38. 7
June 13	1, 700	1, 500	58. 2	48. 1
1924				
Apr. 8	1, 400	1, 400	73. 3	69. 6
Aug. 19	200	600	63. 5	70. 3
1926				
Sept. 9	900	600	63. 4	57. 3
Sept. 16	450	600	78. 0	75. 3

the average percentage settling in the afternoon was smaller than in the morning of the same day. The average difference in these 6 cases amounted to about 5 percent of those transferred.

CROWDING

The percentage that settled depended to a slight extent upon the number of crawlers transferred to the plant. The mean number settling and the standard deviation from the mean were compared when 50, 100, 200, and 300 were transferred on September 9, 1926. With only 50 nymphs the variation was excessive, but with 300 there seemed to be some loss from crowding on the 1-year-old seedlings used in this experiment.

SUMMARY

These studies deal with the productivity of the camphor scale and its biology from the time of egg deposition to the settling of the newly hatched nymphs, the period in which the maximum abundance of the succeeding generation is determined.

The productivity of the females in the laboratory was estimated in two ways, by counting the newly emerged scales on potted camphor-tree and Satsuma orange plants, and by removing the covering from females on camphor-tree twigs and observing oviposition directly. By the first method 13 first-brood females on camphor-trees produced an average of 125 offspring, and 24 second-brood females produced an average of 110. Productivity on Satsuma orange was somewhat higher. By the second method the females showed a higher total reproductivity than that estimated from emergence records. Between 14° and 29° C. the rate of oviposition increased with rising temperature according to logarithmic curves, but temperatures both below and above these limits retarded egg laying.

Productivity in the field was determined by counting the eggs beneath field-collected adults. The oviposition period ranged from 13 weeks for the overwintered females to 5 or 6 weeks for the first- and second-brood females.

The time required for the eggs to hatch varied widely with the age of the female. Eggs from the youngest females had the longest incubation period, but the older females retained their eggs longer after development had started. In most cases high temperatures shortened the embryonic period.

The crawlers showed a marked diurnal periodicity in the time of emerging from beneath the covering of the parent scale. They seldom came out between 6 p. m. and 6 a. m. The time of emergence during the day depended in part upon the temperature, being earlier on the warmer days. In midsummer the majority had emerged before 11 a. m. When midday temperatures fell below 20° C., few crawlers came out at any time during the day.

The crawlers were positively phototropic, but orientation toward a light source was not exact. The rate of crawling diminished as the angular departure from the light source increased, and was accelerated by high temperature within the range 17° to 37° C.

In experiments under controlled conditions the highest percentage of crawlers settled in the temperature range 27° to 30° C., but at all temperatures between 22° and 32° C. more than 90 percent of the crawlers that survived settled in the first 6 hours after being transferred to the twigs.

In insectary experiments each degree rise in temperature between 15.2° and 29.2° C. tended to increase the number of crawlers settling and forming covers by 3.5 to 4 percent. Since emergence is also suppressed by low temperatures, it is probable that settling in the field would seldom average below 40 percent of normal emergence.

The crawlers were easily dislodged by air currents. When infested plants were exposed intermittently to a maximum air velocity of 12.7 miles per hour, only 37 percent of the crawlers transferred settled, as compared with 76 percent on plants kept in still air.

Factors of lesser importance in their effect on crawler survival were type of host plant, light intensity, time of transfer, and number of crawlers per plant.

On camphor-trees most of the females settled on the stem and most of the males on the leaves. Of those on the stem, the majority settled around nodes and bases of petioles, while those on the leaves were usually found next to the midrib. The proportion of males and females settling on the leaf and stem was more nearly equal when settling took place in the dark, but the difference was not sufficient to explain the sex difference in location on the plant.

BIOLOGY OF THE CAMPHOR SCALE AND A METHOD FOR PREDICTING THE TIME OF APPEARANCE OF STAGES IN THE FIELD¹

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INTRODUCTION

In a previous paper the reproduction of the camphor scale (*Pseudonidia duplex* Ckll.) and the behavior of the crawler stage were discussed (1).² The present paper is concerned with the later development of the insect, and includes the biology from the time of settling to the reproductive period, largely as determined from insectary experiments. It also deals with the seasonal history of the scale in the field and gives a method for predicting the time of appearance of each stage, which is useful in the timing of applications of insecticides so that they will be most effective.

METHODS USED IN BIOLOGICAL STUDIES

All the experiments were conducted in a screened insectary, where the scales were not exposed to direct sunlight or to rains. Potted camphor-tree plants 8 to 10 inches high were used as hosts in all the experiments on development. Material was taken from infested camphor-trees in the field. The scales, eggs, and crawlers were removed from the twigs to paper, and the crawlers transferred to the host plants with fine camel's-hair brushes. Only those nymphs that moved toward the light were used. During the transfer and settling of the crawlers the plants were shielded from air currents by glass frames.

Before a plant was infested, an ink line was drawn down the stem and a diagram made of the figure that would be formed if the stem were split on this line and the two halves, outer surface up, laid side by side, the leaves being drawn separately and numbered. Before the first molt the locations of the scales were indicated on this diagram and each was given a number. The individual scales could then be followed as long as they remained on the plant.

The number of insects that had formed covers at the end of 48 hours was taken as the number that had settled. Records of the length of each stage of the females were taken every day within about 2 hours of noon. In the case of the last three molts of the males usually 10 individuals were examined at 2-day intervals, but in cool weather the intervals between observations were sometimes longer. Daily mean temperatures were obtained by planimeter readings from thermograph records (checked against calibrated thermometers) on a 12-noon basis. Experiments were started with the first brood of the year and

¹ Received for publication Nov. 5, 1934; issued April, 1935.

² Reference is made by number (italic) to Literature Cited, p. 283.

continued through the last brood in order to cover as wide a range of temperature as possible.

DESCRIPTION OF MOLTS

The female molts twice. Preceding each molt her body becomes swollen and its contents fluid, and the integument sticks to the covering. The time of molting can be detected by a color change in the covering. For several days previous to the molt this covering has a transparent amber color; then, as the molt takes place it takes on a

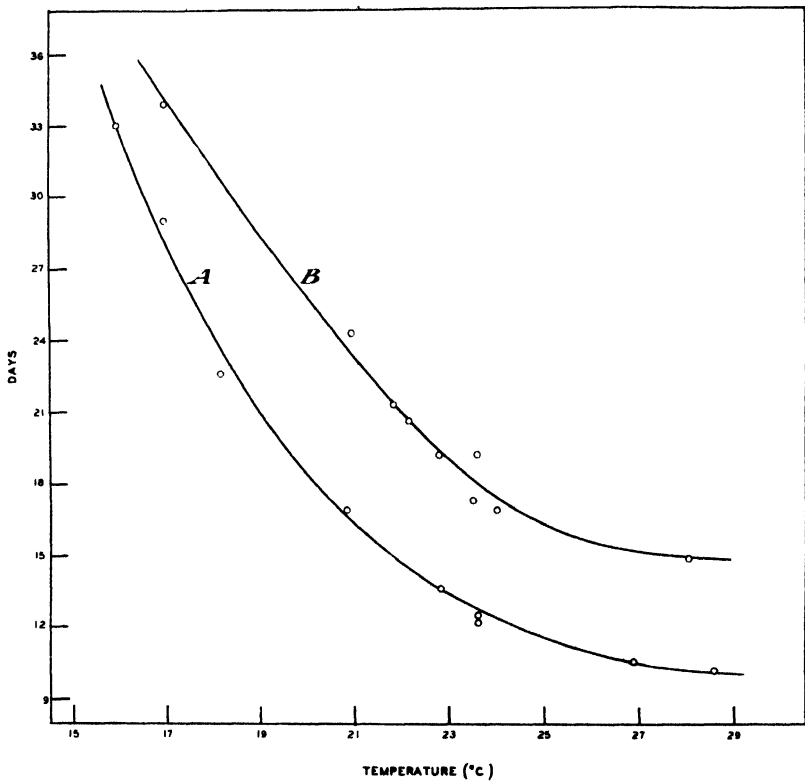


FIGURE 1.—Length of the first (A) and second (B) stadia of the camphor scale (*Pseudonidia dupler*) as functions of the mean temperature.

drier, brighter appearance and loses its transparency. If the adult female is fertilized, the eggs are laid and hatched beneath the covering. The male is identical in appearance with the female when in the first larval instar, but in the second instar he becomes elongated and the covering is oblong-elliptical. The male has four larval instars. The fourth molt brings him into the winged adult stage, after which he emerges from beneath the covering and is ready to mate. The last three molts cannot be distinguished by color changes and the covering must be removed to ascertain the stage of the male beneath. The insect does not survive this treatment.

RATE OF DEVELOPMENT

The relation between the length of the first stadium (males and females) and temperature is shown in curve *A*, figure 1, where each point represents the mean for the scales on a group of plants infested at the same time. The length of this stadium was calculated from the time the scales were set out on the plant, since it had been previously shown (1) that most of the crawlers settle within a few hours after being transferred. The number of scales in each group ranged from 326 to 726, and there were 4,771 scales in all. The mean temperature

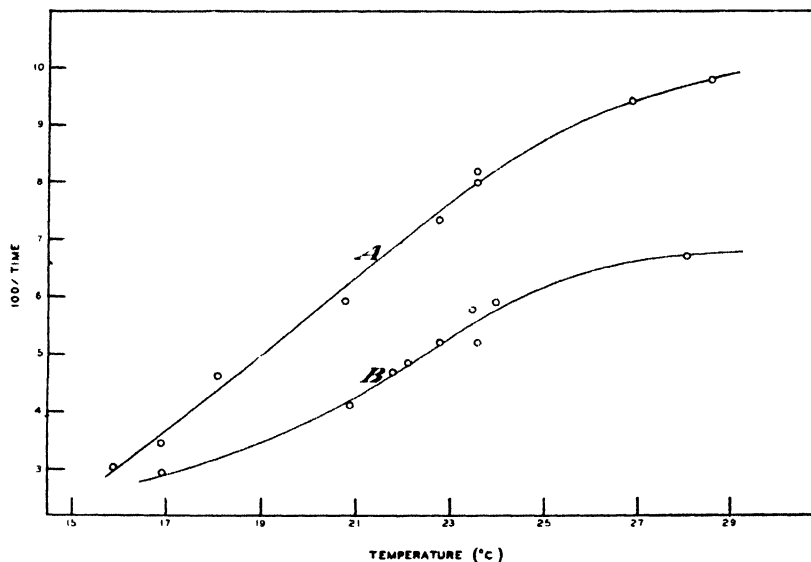


FIGURE 2.—The rate of development of the camphor scale in relation to temperature. *A*, During the first stadium, *B* during the second stadium.

ranged from 15.9° to 28.6° C. and the mean length of the stadium from 10.2 to 33 days. The numerical data are given in table 1.

TABLE 1.—Length of the first stadium of camphor scales (both sexes) on potted camphor-tree plants in the insectary at different temperatures

Brood	Date started	Scales	Average length of stadium	Mean temperature	Brood	Date started	Scales	Average length of stadium	Mean temperature
		<i>Number</i>	<i>Days</i>	<i>° C.</i>			<i>Number</i>	<i>Days</i>	<i>° C.</i>
First.....	Mar. 7	326	33.0 ± 0.04	15.9	Second.....	June 17	726	10.2 ± 0.01	28.6
	Mar. 18	456	29.1 ± .03	16.9	Third.....	Sept. 2	549	10.6 ± .02	26.9
	Mar. 27	439	22.6 ± .04	18.1		Sept. 15	582	12.5 ± .02	23.6
	Apr. 11	686	16.9 ± .02	20.8		Oct. 2	565	13.6 ± .02	22.8
	Apr. 30	442	12.2 ± .01	23.6					

The usual method of measuring relative growth velocities is by the reciprocal of the time required to complete a given stage. These values for different temperatures are shown in figure 2, *A*. The curve is approximately a straight line between 16° and 23.5° C., but beyond the higher point the effect of change in temperature on developmental

velocity decreased. Whether the decrease was due to the inclusion in the means of temperatures above 30° C. cannot be determined from these data.

A study of the statistical constants covering the relation of the length of the stadium to the temperature in these experiments showed that variables other than temperature had a negligible effect upon the rate of development. Curve *A* in figure 1 was fitted to the means by freehand graphic approximation, the algebraic sum of the residuals being made zero, and the difference between this curve and the observed individual record was read off for each case. The index of correlation was then calculated from the formula—

$$r_{yx} = \sqrt{1 - \frac{\sigma_{zy}^2}{\sigma_y^2} \left(\frac{n-1}{n-m} \right)} \quad (4)$$

This gave a value of 0.98, showing that the variation in length of the first stadium was almost entirely a function of temperature. Although the maximum time over which molting extended for any group was 7 days, the majority of scales molted closely around the mean date. In no case had as many as 10 percent molted 2 days previous to that time. No significant variation in the length of the stadium was found which depended on sex or position on the plant.

The results for the second-instar females are given in table 2 and figure 1, *B*. The second stadium is shown to be somewhat longer than the first at corresponding temperatures. In figure 2, *B*, the reciprocals of the time have been plotted against temperatures. No portion of this curve is a straight line. The index of correlation for 1,301 cases was 0.96. The maximum variation in length of the stadium for any group was 9 days, but again in no group did 10 percent of the scales show a 2-day variation from the mean.

TABLE 2.—Length of second stadium of female camphor scales on pollarded camphor-tree plants in the insectary at different temperatures

Brood	Date majority made first molt	Scales	Average length of stadium	Mean temperature	Brood	Date majority made first molt	Scales	Average length of stadium	Mean temperature
First.....		<i>Number</i>	<i>Days</i>	<i>° C.</i>	Second.....		<i>Number</i>	<i>Days</i>	<i>° C.</i>
	Apr. 9.....	71	24 3±0.09	20.9		June 27..	259	14 9±0.04	28.0
	Apr. 16..	171	21.3±.06	21.8		Sept. 13	134	19.2±.04	23.6
	Apr. 19..	113	20.6±.07	22.1		Sept. 27..	138	19.2±.06	22.8
	Apr. 28..	211	17.3±.04	23.5		Oct. 15..	67	33.9±.06	16.9
	May 12..	137	16.9±.03	24.0					

Variation in the length of both the first and second stadia was greatest in the scales of the first brood, as would be expected from the lower temperatures prevailing. Since in this brood the temperature at the time of the second molt was higher than at the time of the first molt, those scales that completed the first molt early were exposed to a lower mean temperature during the second stadium than those molting later. Consequently, there was an apparent tendency for scales making the first molt earlier or later than the average to compensate for this difference in the second stadium. When the length of the second stadium for the individual scales in the first two

groups was associated with the length of the first stadium, regression coefficients of -0.54 day and -0.53 day were obtained. When the differences in individual mean temperatures were considered, by correlating the difference between the expected length of the first stadium, predicted from figure 1, *A*, and the observed length with the equivalent departure in the second instar, the regression coefficient was not significant in one group, and in the second it had the small, though significant, value of -0.12 day. Although the first correlation was largely spurious, similar temperature conditions obtained in the field, and its existence in the case of the brood showing the greatest variation is further justification for using mean periods in field prediction. To use the minimum length of the stadium would introduce a systematic error, since those individuals in which the first stadium was shortest tended to spend a longer time in the next

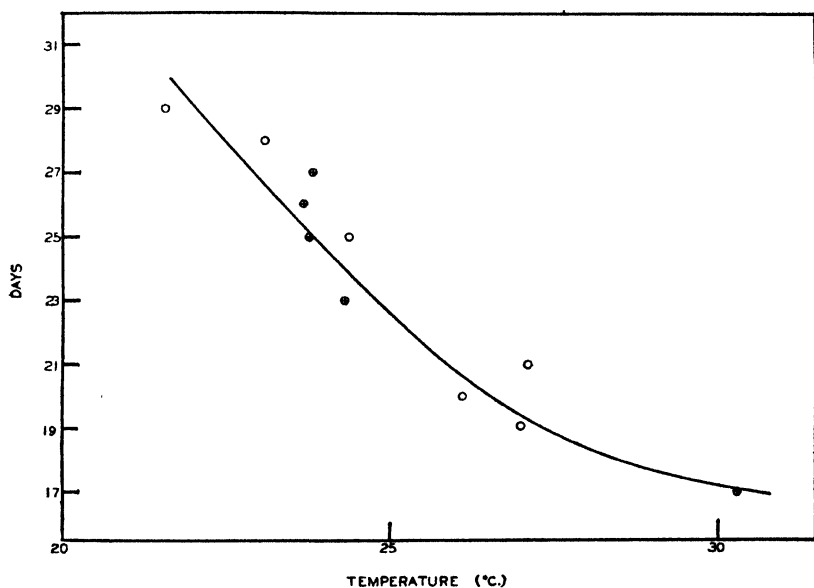


FIGURE 3 Length of preoviposition period of the camphor scale in relation to temperature. Field data are shown by plain circles, insectary data by circles with crosses.

stadium than those that made the first molt at later dates. Peairs' (6) method of plotting minimum periods therefore has not been used.

The time from the second molt to the production of the first egg, or the preoviposition period, is shown in figure 3 and table 3. Because of the limited temperature range of the insectary studies, the data from the field counts have been included. Each entry represents a group of plants infested on the same day in the insectary studies or a brood in the field counts. Since it was necessary to depend on periodic removals for the insectary data, the results can be considered only as approximate. An additional source of variation lay in the fact that the exact date of fertilization was unknown. Therefore, the time the first females started to lay probably was the best indication of the end of the preoviposition period in the field, since these females were presumably the first to be fertilized. The data from both sources showed fair agreement.

TABLE 3.—Length of preoviposition period of the camphor scale at different temperatures as estimated from field and insectary data

Source of data	Mean temperature	Average length of period	Source of data	Mean temperature	Average length of period
	° C.	Days		° C.	Days
Field	21.6	29	Insectary	23.7	26
	23.1	28		23.8	25
	24.4	25		23.8	27
	26.1	20		24.3	23
	27.0	19		30.3	17
	27.1	21			

The time from the beginning of reproduction to the emergence of crawlers is shown in figure 4 and table 4. Because of the short

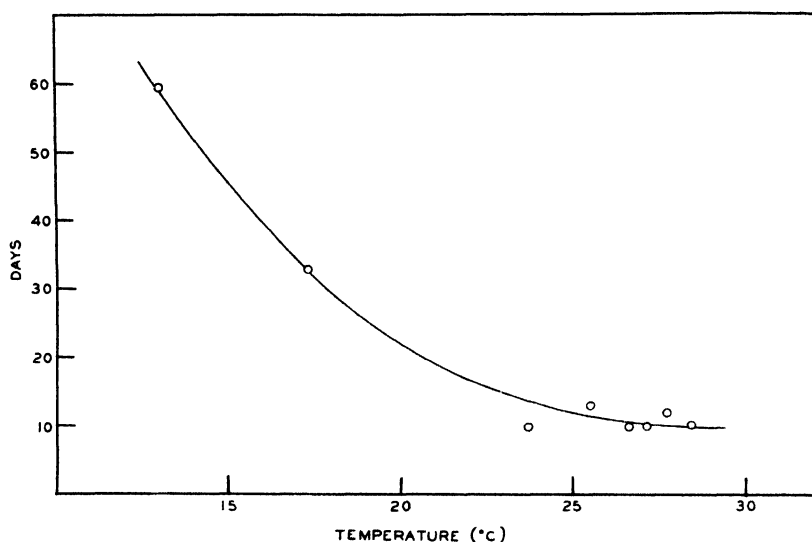


FIGURE 4.—Time from the beginning of egg production of the camphor scale to appearance of newly settled nymphs in relation to temperature. Each circle represents the period for a single brood as observed in the field.

interval between oviposition and hatching, and between hatching and emergence, the period from beginning of oviposition to emergence of young proved to be a more reliable interval than the two short periods. These records were taken entirely from the field counts.

TABLE 4.—Period from appearance of first eggs to emergence of crawlers of the camphor scale at different temperatures, as estimated from field counts

Mean temperature	Average length of period	Mean temperature	Average length of period
° C.	Days	° C.	Days
13.0	59	26.6	10
17.3	33	27.1	10
23.7	10	27.7	12
25.2	13	28.4	11

Males of the first instar showed no difference from the females. For the later stages periodic examinations were made as previously described. The number of days from the first molt to the time the majority had reached each succeeding stadium was determined, and the lengths of the stadia were calculated from these figures. Since only a small number were removed each time, and the last three stadia were of short duration, these results also were only approximate. The data are summarized in table 5.

TABLE 5.—Length of later stadia of male camphor scales at different temperatures ¹

Second stadium		Prepupal stadium		Pupal stadium		Adult (until emergence)	
Temperature	Length	Temperature	Length	Temperature	Length	Temperature	Length
° C	Days	° C	Days	° C	Days	° C	Days
21.0	25	19.7	3	15.9	15	13.6	3
21.9	22	21.7	3	23.2	5	22.6	5
22.2	20	23.0	3	23.4	1	23.5	3
22.8	19	23.7	3	23.9	5	23.8	1
23.6	18	24.0	3	24.1	5	24.1	3
23.6	18	24.9	2	24.5	6	24.4	4
24.0	16	25.2	2	24.8	4	25.0	3
28.1	16	26.2	2	26.6	3	27.0	3

¹ 1 for length of the first stadium see table 1.

MORTALITY

The mortality for the first-instar females on the stem ranged from 5.9 to 33.1 percent, and for the second instar from 30.1 to 47.0 percent, and the percentage of adults that died without reproducing ranged from 23.8 to 47.6. If we consider the totals of all the experiments, the proportions that died during the three stadia calculated as percentages of those that entered the stadium were 11.0, 34.9, and 38.7 percent, respectively. The proportions of those settling that survived each stadium were 89.0, 56.4, and 33.4 percent, respectively. These figures have been corrected for scales killed by parasites and predators and those removed for observation. No relation between mortality and temperature or density of population was evident in these experiments. In the field, where the infestation is much heavier at times, population density is a factor, and subfreezing temperatures may kill some scales.

The mortality of the first-instar nymphs was somewhat higher on the leaves than on the stem. The removal of immature scales following the first molt, and the twisting and handling of the leaves during the examination, with the consequent loosening of the scale covers, made the figures on later mortality of doubtful value.

SEXUAL DIFFERENCES IN PLACE OF SETTLING AND RATIO OF SEXES

On camphor the two sexes show a marked difference in settling on different portions of the plant (table 6), the males generally going to the leaves and the females to the stem, although this characteristic has been observed to vary on different plants. Generally the males are found on the leaves, but the females on some plants settle on the leaves as readily as on the stem.

TABLE 6.—*Percentage of each sex of the camphor-scale nymphs settling on different parts of potted camphor-tree plants*

Sex	Total scales	Nymphs settling on—				
		Stem	Leaf blades		Petioles	
			Upper surface	Lower surface	Upper surface	Lower surface
Female.....	<i>Number</i> 3, 050	<i>Percent</i> 75.8	<i>Percent</i> 4.6	<i>Percent</i> 12.6	<i>Percent</i> 3.1	<i>Percent</i> 3.9
Male.....	3, 296	4.1	53.0	28.9	5.3	8.7

The sex was known only of those scales that survived well into the second stadium. On the basis of such scales, the proportion of males was 48.2 percent and of females 51.8 percent. Since the two sexes settled on different parts of the plant, these figures would not represent the true sex ratio if there was a difference in mortality before the sexes became distinguishable. It may be assumed, however, that the data on the scales of known sex do represent the true proportion of the sexes which settled on each part of the plant. If the total number that settled on each part is multiplied by the percentage of each sex later found, to give the true numbers of males and females in the newly settled nymphs, and the products are converted into percentages, we obtain males 51.9 percent, females 48.1 percent. The sex ratio, then, was practically unity.

FERTILIZATION

Fertilization is necessary for egg production. In a large series of experiments no parthenogenesis has been found. Unfertilized females never take on the purple-brown color characteristic of the gravid female. In cages 1 male has fertilized as many as 3 females, and it is possible that in the field the male can fertilize a still greater number. The female has also been observed to mate more than once.

SEASONAL HISTORY AND PREDICTION OF TIME OF APPEARANCE OF STAGES

METHODS OF PREDICTION

The two methods that have been used most by American entomologists for predicting the length of developmental stages are the "day-degree", or accumulated-temperature, method and the "developmental-unit" method.

The first method has been applied by Glenn (5) to data on the codling moth. It is based on the old idea that the time-temperature curve is an equilateral hyperbola, or that the developmental velocity-temperature curve is a straight line. Therefore, the product of time and effective temperature—i. e., degrees above the hyperbolic zero—will be a constant, so that when the number of day degrees required to complete the different stages has once been determined, it should

be possible to predict the length of the stages from the accumulated temperatures. It is doubtful whether the velocity-effective temperature curve is really a straight line over any part of the temperature range, but in many cases it is approximately so in the middle of the range. Glenn corrected for deviations at high, but not low, temperatures.

Shelford (8) discusses Glenn's work in some detail, and then develops a method that appears to be similar in principle although introducing more refinements. He uses the "developmental unit", which he defines as "the difference in amount of development produced in one hour by a difference of one degree of mean medial variable temperatures (other conditions being average)." "Medial temperature" refers to the straight-line (or approximately straight-line) portion of the velocity curve. This developmental unit, however, is a variable, the value of which depends on the climatic conditions, such as falling or rising temperatures or rainfall. Shelford makes corrections for high and low temperature, humidity, rainfall, etc. His use of hourly instead of daily temperature is a theoretical improvement, since there is less variation during the shorter period. On the whole, however, the method appears to be complicated and laborious as to both the fundamental experimentation necessary and the application to field prediction. Any method based on the assumption of a straight-line velocity curve requires so many corrections for accurate work that it is desirable to introduce a method that does not depend on such an assumption. For example, the velocity curve (fig. 2, *B*) for the second-instar females, as discussed on page 270, has no portion that is a straight line.

The method herein proposed is based on no assumptions whatever beyond that necessary to any prediction of this kind, that in the absence of other modifying external factors the development of an insect will repeat the same response to the same temperature. With the camphor scale a sufficient basis for prediction of the time of appearance of the different stages is the number of days required to complete a stage at any mean temperature, as shown in figures 1, 3, and 4. To get the expected date of appearance of any instar, a running average is kept of the mean daily temperatures until a temperature-time combination is reached which would permit completion of the stage.

This method may be illustrated with a prediction of the beginning of the second stadium of the first brood in 1926 (table 7). The first newly settled nymphs were observed on March 30. Beginning with this date, a running average was kept of the mean daily temperatures, and on the day (about Apr. 21) when this average approached a value that was indicated on curve *A* of figure 1, the number of days since March 30 was compared with the number of days corresponding to that temperature on the curve. This comparison was then repeated every day until the two periods were of the same length. The data given in table 7 indicate that the second instar should appear on April 25. The time of completion of the second stadium would then be calculated from this date and the readings made from figure 1, *B*.

TABLE 7.—Data for prediction of date of appearance of the second instar of the first brood of camphor scales in 1926, when newly settled nymphs were first observed on Mar. 30, 1926

Date		Length of period from Mar 30	Average daily mean temperatures from Mar. 30	Length of first stadium as obtained from fig 1, .1
		Days	° C.	Days
Apr 21	-----	22	16.8	29+
22	-----	23	17.0	28
23	-----	24	17.1	27+
24	-----	25	17.3	26.5
25	-----	26	17.4	26

† Agreement of numbers in italics indicates that the second instar may be expected to appear on Apr. 25.

The most probable source of error with this method lies in the averaging of temperatures on the basis of a time period instead of a fraction of a developmental period. If an insect spends 5 days at 28° C. and 5 days at 20° C., the mean temperature, calculated on a time basis, would be 24° C. But if the rate of development is twice as rapid at 28° as at 20° C., the portion of the stage passed at 28° would be twice as great as that at 20° C. This error, however, is less under a continuously varying temperature than where the insect is under one temperature for a certain period and is then suddenly changed to a very different temperature. The fact that the data on which the predictions are based were obtained under conditions similar to those in the field further reduces this error. The method is simple, direct of application, and should be capable of extension to many other insects.

SEASONAL-HISTORY DATA

Counts were made of scales on a row of camphor-trees in Audubon Park, New Orleans, at weekly intervals during 1926, 1927, and 1928. No thermograph records were kept, but the United States Weather Bureau maintains a substation in the park which records maximum and minimum temperatures. These temperatures were compared with the main Weather Bureau station extremes for New Orleans and the Weather Bureau means (computed on an hourly basis) modified accordingly to obtain the mean park temperatures. Since the maximum and minimum temperatures in the park were rather erratic as compared with the official temperatures, a constant rather than a day-to-day correction was applied. The mean park temperatures were consistently lower than the readings of the main Weather Bureau station, the average deviation for the period from the first appearance of newly settled nymphs to the maturing of the last brood being 1.0°, 0.9°, and 1.2° C., respectively, for the 3 years. Therefore, the daily mean temperatures given in the monthly summary of the Weather Bureau were reduced 1.0° C. for calculating the mean temperatures under which the scales developed. This illustrates the necessity of checking habitat temperatures of an insect against Weather Bureau records in order to allow for consistent departures.

Scales were recorded according to instars, and the adults were further divided into white, pink, gravid, and reproducing. The

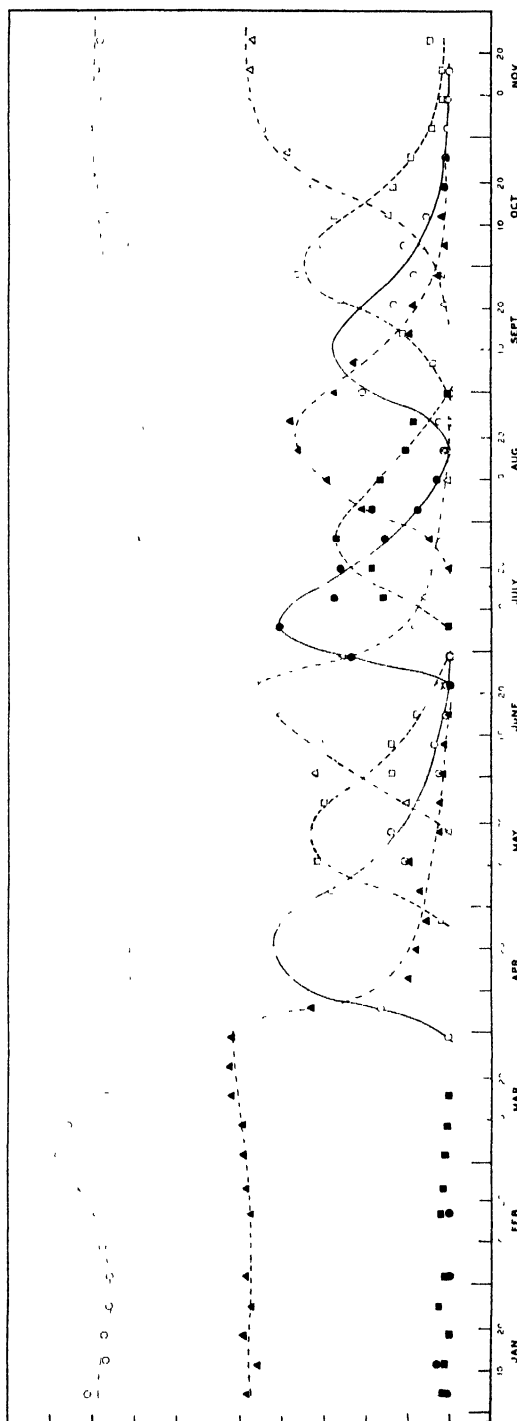


FIGURE 5.—The lower curves show the succession of stages of the camphor scales over an 11-month period in 1928. Circles represent the first instar, squares the second instar, and triangles the third instar. Outline and solid figures distinguish between successive generations. The ordinates are the percentages of the total living population in each stage. The upper curve shows the mortality over the same period, ordinates being the percentages of the total count that are dead.

numbers of living and dead individuals of each stage were tallied, with the dead separated into those killed by parasites, predators, freezing, and other natural causes. In addition, the number of eggs and crawlers under each reproducing female was recorded. Usually one twig was taken from each of four trees, and approximately 500 scales were counted from these twigs. Only the female scales are discussed here, the assumption being made that all the first instar and early second instar were females. As is seen from the proportion of sexes settling on the stem in the insectary, the error introduced is small.

When the beginning of a stage fell between the weekly counts, it was necessary to extrapolate the curves to the origin. Such curves were compared with those for other years to determine their most probable position. Except in the case of the second- and third-brood adults, the broods were separated with little difficulty. There was some question as to the time the third-brood adults first appeared, although when they were present in any numbers they were easily distinguished.

A graph on which the first year's counts are recorded is shown in figure 5. This is typical of the data for the other years, which were plotted with smoothed curves in the same way. As would be expected, the curves have a tendency to flatten in successive generations, and the tails on the curves are most pronounced in the third generation.

These counts present a picture of the scale population throughout the year. Their most important use is for checking the applicability of insectary data for prediction of the succession of stages in the field. They also show the incidence of parasites and, to some extent, of predators, and the effect of climatic conditions on mortality.

PREDICTION OF STAGES

In predicting the time of appearance of the different stages, the writers have worked with the time from the first of one stadium to the first of the next, for to figure from peak to peak would require that the two stadia involved be of the same length. They have started with the appearance of newly settled nymphs in the spring and checked the expected date of the appearance of each stage, calculated as described in the example given above, against the observed appearance of the stage in the field.

A comparison of the expected and observed dates for the 3 years is shown in table 8. On the whole the agreement is close, justifying the method. There are two serious deviations. One is a difference of 5 days for the first brood, second instar, in 1927. The fact that the expected dates tend to run later than those observed for this year indicates that the first newly settled nymphs in the field were missed in the counts. The second serious discrepancy is for the third brood, third instar, in 1926. Little importance can be attached to agreement or disagreement between observation and calculation at this point, because of the difficulty of ascertaining the actual beginning of this stage. As to the other discrepancies, a difference of a day is well within the variation of the material, and since counts were made at weekly intervals, the extrapolation sometimes necessary may be in error by a day or so.

TABLE 8.—Comparison between observed and predicted dates of appearance of different stages of the camphor scale in the field

Brood and stage	1926			1927			1928		
	Expected date	Observed date	Temperature	Expected date	Observed date	Temperature	Expected date	Observed date	Temperature
			° C.			° C.			° C.
First brood:									
First instar		Mar. 30			Feb. 21			Mar. 19	
Second instar	Apr. 25	Apr. 26	17.4	Mar. 27	Mar. 22	15.7	Apr. 16	Apr. 19	16.8
Third instar	May 19	May 18	20.7	Apr. 17	Apr. 16	21.8	May 12	May 9	19.8
Second brood:									
Eggs	June 12	June 12	24.6	May 17	May 15	21.7	June 6	June 6	23.4
First instar	June 21	June 22	27.2	May 29	May 26	24.6	June 18	June 20	25.2
Second instar	July 3	July 5	26.8	June 9	June 7	26.6	June 29	June 29	26.5
Third instar	July 18	July 19	26.9	June 24	June 21	26.0	July 14	July 16	27.3
Third brood:									
Eggs	Aug. 7	Aug. 8	26.8	July 13	July 11	27.1	Aug. 4	Aug. 6	26.2
First instar	Aug. 17	Aug. 17	26.8	July 23	July 23	27.8	Aug. 14	Aug. 16	28.2
Second instar	Aug. 27	Aug. 27	27.6	Aug. 3	Aug. 3	26.4	Aug. 24	Aug. 27	27.9
Third instar	Sept. 11	Sept. 16	27.3	Aug. 18	Aug. 19	27.1	Sept. 8	Sept. 11	25.8

† The third-instar females are adults.

The agreement between the expected and observed results, therefore, is well within the accuracy of the data. To facilitate prediction

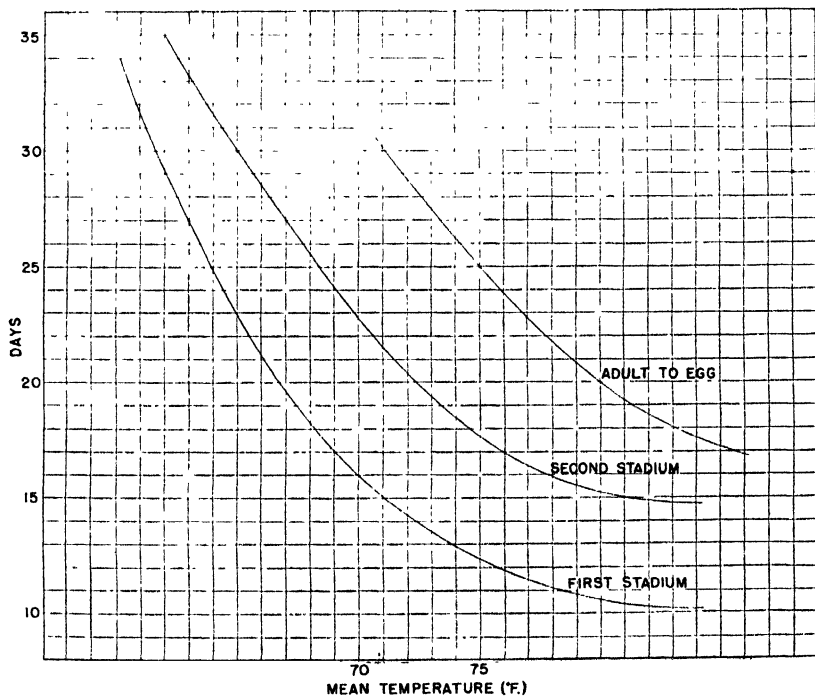


FIGURE 6.—Length of first and second stadia of nymphs and of preoviposition period of adults of the camphor scale as functions of the mean temperature. Curves of figures 1 and 3 converted to Fahrenheit scale for use in prediction directly from Weather Bureau records.

directly from Weather Bureau records, the curves of figures 1, 3, and 4 have been reproduced in figures 6 and 7 with the temperature on the Fahrenheit scale.

In the preceding discussion the prediction of the appearance of eggs laid by overwintering females or of the first instar of the first brood from the temperature records has not been considered. If this could be done, there would be no necessity for making field observations, and prediction could be made entirely from the weather records.

An attempt was made to predict the appearance of eggs on the basis of mean temperature from an arbitrary date (Dec. 1) to the beginning of oviposition in the winter, based on seasonal-history data over the 5 years 1924-28. The points for 4 years fell on a smooth curve, but the date for 1924 was consistently earlier whether the point selected was first increase in production, 2, 5, or 10 eggs per female. That the reactions of the overwintering females differ from those of the preceding brood is shown by the fact that, if this brood gave the same response

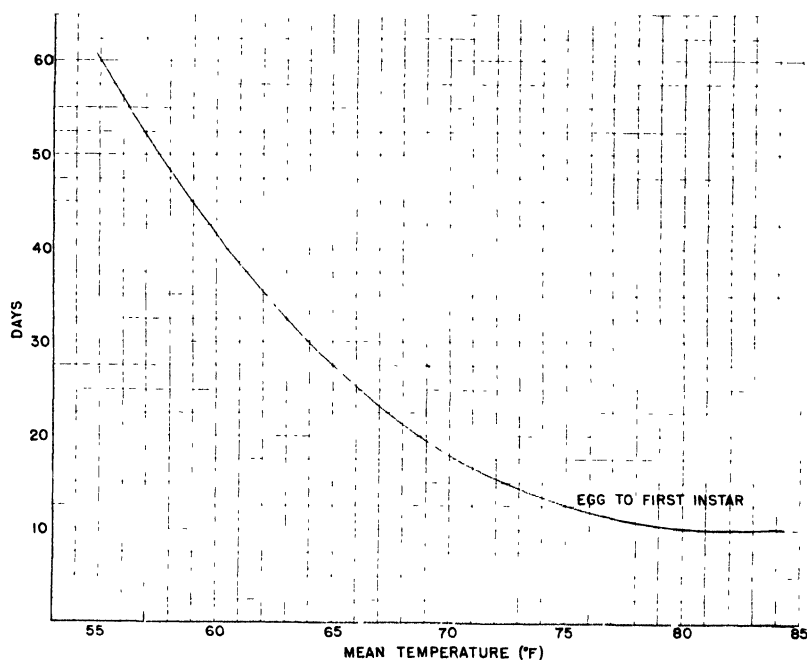


FIGURE 7 —Length of period from beginning of egg production to appearance of newly settled nymphs as a function of temperature. (Curve of figure 4 converted to Fahrenheit scale for use in prediction from Weather Bureau records.)

to temperature as the others in the 3 years (1926, 1927, and 1928) in which complete counts are available, eggs of another brood would have been produced each fall, whereas only in 1927 was there a partial fourth brood. In 1925, when the first adults of the third brood appeared about August 22, there were also a small number that reproduced. In both cases production, instead of gradually increasing, showed a tendency to become stationary and then decline during December and the first part of January. In 1923, 1924, 1926, and 1928 no fourth-brood nymphs were found. It is, therefore, probably not justifiable to select an arbitrary date, since oviposition in winter depends in part on the time of maturation the preceding fall. This is

shown by the following data from insectary experiments in which the second molt was completed at different times:

Date majority made second molt:	Date of first eggs
Oct. 2.....	Feb. 12
Oct. 16.....	Feb. 20
Nov. 18.....	Apr. 18

Complete temperature records on the plants involved are not available, but all were subject to the same conditions.

Until the reactions of the overwintering females are better understood, therefore, it is necessary to base predictions on the beginning of some nonoverwintering stage. The appearance of newly settled nymphs is preferable to the beginning of egg production, for several reasons. Since a few eggs are present throughout the winter, the date of the first continued increase in egg production is more uncertain than the appearance of newly settled nymphs. Furthermore, to determine the beginning of increased egg production may require counts over an extended period, while the newly settled nymphs are easily recognized and the date of their appearance can be ascertained with little difficulty.

FACTORS AFFECTING MORTALITY

The interpretation of the mortality figures is obscured by the fact that the scales often remained on the twig for some time after death, and the time of death was therefore unknown. In addition, since young were being produced during a large part of the time, successive counts were samples of a population whose make-up was constantly changing. For these reasons the figures represent only the portion of the population dead at the time of counting, and thus cannot be used to ascertain the proportion of those entering a given stage which failed to survive.

The mortality in 1926 is shown in the upper curve in figure 5 where, to permit comparison with the living scales, the same abscissas have been used in both cases. The mortality tended to reach a maximum just before the start of the new brood and a minimum at about the peak of the first instar. At these times the scales had been exposed to the maximum and minimum chances of death, since the average age of the scale population would be greatest at the first point, and least at the second.

The percentage dead in the overwintered brood was lower in 1928 than in 1926 or 1927, as a result of decreased parasitization; otherwise, there was little difference in the 3 years.

During the time in which mortality records were taken, there were 4 years in which the temperature fell below -5°C . In January 1924 minimum temperatures of -3.3°C ., -7.2°C ., and -2.8°C ., respectively, on 3 successive days killed about 16 percent of the scales (2). In December 1925, when the temperature fell to -5.6°C ., approximately 4 to 5 percent of the population were frozen. About the same mortality was experienced in January 1928 with minimum temperatures of -5.6°C . and -6.1°C . On February 8 and 9, 1933, when temperatures of -3.9°C . and -6.7°C . were recorded, between 30 and 37 percent of the scales on uninjured wood were frozen (3). In reality the total mortality in 1933 was much higher because most of the younger wood of camphor-trees was killed

back, and this wood bore most of the living scales previous to the freeze.

These records show considerable variation in the mortality resulting from comparable temperatures, the probable reasons for which have been found in examination of the seasonal-history records and weather records preceding the low temperatures. In 1924 and 1933 the months preceding the minimum temperatures showed a daily average excess of 7.9° and 5.8° C., respectively. In 1925 and 1927 the preceding months showed a daily average deficiency of 3.3° and 1.5° C., respectively. In the 2 years when subfreezing temperatures were preceded by subnormal temperatures, there was a much lower mortality than in those years when freezes were preceded by temperatures well above the normal. In all years except 1933 the scales were in the adult stage but had not started oviposition when the subfreezing temperatures occurred. In 1933 nearly 95 percent of the scales were reproducing, and the highest mortality was experienced, although the temperature did not go so low as in 1924. The records indicate that subfreezing temperatures are fatal to more scales when they have been preceded by warm weather than when they follow a period of subnormal temperature, and have greater effect the more advanced the development of the scale population.

The number of scales parasitized never assumed significant proportions except in the third broods of 1926 and 1927 and the over-wintered brood of 1926 (third brood of 1925). In these cases the parasitization was approximately 20 percent. This decreased rapidly with the emergence of the first brood, and during the spring and summer months seldom were more than 3 percent of the scales parasitized. That the increases in the third brood were not merely the result of the parasitized scales from the preceding broods remaining on the plant is indicated by the fact that in the second brood no increase over the first brood was noted, and that in 1928 the parasitization of the third brood was comparable to that in the earlier broods of that year.

The scales were usually killed in the second instar, but occasionally parasitized adults were found. The following parasites have been recovered: *Aphelinus fuscipennis* Howard, *Aspidiotiphagus citrinus* (Crawford), *Prospaltella aurantii* Howard, *P. fasciata* Malenotti, and *Signiphora flavopalliat*a Ashmead. *Metataptus torquatus* Malenotti has also been taken from cages, but this may be an egg parasite of Psocidae.

From these counts little can be said about the efficiency of predators, since the scale is usually completely removed when attacked. A bagworm, *Platoceticus gloveri* (Packard), has been locally quite effective, and trees have been observed that were practically cleaned of scales by this insect (?). Two species of lady beetle, *Chilocorus bivulnerus* Mulsant and *Microvelia misella* Leconte, also feed on the scale, but none of these natural enemies has been sufficiently effective to be relied upon as a means of control.

SUMMARY

The biology of the camphor scale (*Pseudaonidia duplex* Ckll.) from the time of settling to the beginning of the reproductive period has been studied in the insectary and field.

The rate of development was found to be dependent largely upon the mean temperature, the index of correlation between the length of the first stadium and the temperature being 0.98, and for the length of the second stadium, 0.96. Curves are given for the length of the first and second stadia, the period from the second molt to egg production, and the time from the first egg to appearance of newly settled nymphs, as functions of temperature.

Of the females that settled on the stem, 88.9 percent completed the first stadium, 56.4 percent the second stadium, and 33.4 percent reproduced.

Approximately equal numbers of males and females were produced, and fertilization was necessary to reproduction.

The mean temperature from the date of emergence of the first brood was found to be a satisfactory basis for predicting the time of appearance of the different stages under field conditions, the calculated and observed dates for field counts extending over 3 years agreeing within the accuracy of the data.

As many as 30 to 37 percent of the scales have been killed by low winter temperatures. Resistance to subfreezing temperatures was greatest when they were preceded by subnormal temperatures and when the scales were in the earlier stages of development.

Parasites and predators have not been important factors in the control of this insect.

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FACTORS AFFECTING INFECTION AND DECAY OF SWEET-POTATOES BY CERTAIN STORAGE ROT FUNGI¹

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INTRODUCTION

Rhizopus soft rot (*Rhizopus nigricans* Ehr. and *R. tritici* Saito), surface rot (*Fusarium oxysporum* Schl.), Java black rot (*Diplodia tubericola* (Ell. and Ev.) Taub.), charcoal rot (*Sclerotium bataticola* Taub.), and fusarium end rot, caused by species of *Fusarium* (including *F. oxysporum*), are the principal storage diseases of sweetpotatoes (*Ipomoea batatas* (L.) Lam.), and they have received major consideration in this paper.³ The factors affecting the invasion of the tissue of sweetpotatoes during storage by fungi other than those listed above have also received some attention.

The object of the present investigation was to study the effect of the following factors on infection and decay of sweetpotatoes by the storage rot fungi just mentioned: Presence or absence of the pathogene, infection and decay by other fungi, amount of inoculum, wounding, suberization, periderm formation, temperature, humidity, and time. These factors will be considered in connection with the various diseases. A study was made also of the effect of temperature on the growth of *Diplodia tubericola* and *Sclerotium bataticola* in culture media. The investigations were made in Washington, D. C., and at the Arlington Experiment Farm, Rosslyn, Va.

REVIEW OF LITERATURE

Rhizopus tritici and *R. nigricans* are unable to attack or penetrate (5, 11) the unbroken skin of the sweetpotato. When roots are wounded these fungi are able to dissolve the middle lamellae (3, 4, 5) of the cells beneath the skin, thus separating the cells and disintegrating the tissues. Hauman-Merck (7) found that *R. nigricans* (*Mucor stolonifer* Ehr.) was unable to pass through cork formed in wounded sweetpotato tissue. Weimer and Harter (13) showed that a wound periderm was an effective barrier against infection of sweetpotatoes by *R. tritici*. They observed periderm formation over a range of temperature from 19.5° to 33° C. at relative humidities ranging from 95 to 97 percent. A periderm was not formed at a low humidity (percentage of relative humidity not given). They also observed that suberization preceded periderm formation.

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³ Although black rot (*Ceratostomella fimbriata* (Ell. and Hals.) Elliott) is a serious storage trouble, it is also a field disease of some importance and in this respect is distinguished from the foregoing storage diseases. Some of the factors affecting its initiation and development in storage have been considered in a previous publication (8).⁴

⁴ Reference is made by number (italic) to Literature Cited, p. 329.

A definite relation has been found between infection by *Rhizopus* (12) (which probably included both *R. tritici* and *R. nigricans*) and relative humidity at a temperature of 23° C.

Artschwager and Starrett (1)⁵ made an extensive study of suberization and periderm formation as influenced by temperature, air humidity, and time. Their work was an outgrowth of the present investigation and was run under the same conditions and in conjunction with some of the infection experiments. The range of temperature at which suberization and periderm formation took place extended from 12.5° to 34.8° C. This lower temperature limit for cork formation is 7° below that obtained by Weimer and Harter (13), who, however, did not employ temperatures between 11.2° and 19.5°. No effort was made to determine the upper limit of periderm formation. In the Artschwager-Starrett experiments periderm formation took place in 3 to 5 days at temperatures above 18° (combined with high humidity), at 18° in 6 days (not included in published data), at 14.7° in 11 days, and at 12.5° only after 25 days. Suberization was found to precede periderm formation, and suberization as well as periderm formation was found to be related to the percentage of humidity at which the roots were stored. At a temperature of 12.5°, suberization and periderm formation were not obtained at a relative humidity below 93 to 94 percent. At 23°, suberization was not obtained below 76 percent relative humidity nor periderm formation below 87 percent within the limit of 10 days. At 28°, suberization occurred at relative humidities ranging from 66 to 95 percent; periderm, however, was not formed within 11 days at 66 percent relative humidity. It will be seen from these data that the higher the temperature the wider was the range of humidities at which both suberization and periderm formation took place.

Wounding and curing (9) have been shown to be important factors affecting infection of sweetpotatoes by surface rot (*Fusarium oxysporum*).

It has been suspected at times that invasion and possibly infection by certain storage-rot organisms may have been secondary to infection and decay by other fungi. Forms of *Fusarium* sometimes invade (2) mottle-necrosis lesions caused by species of *Pythium*. Forms of *Fusarium* also (10) invade lesions produced by such organisms as *Mucor racemosus* Fres., *Botrytis cinerea* Pers., and *Alternaria* sp. at temperatures ranging from 0° to 5° C., when the infected roots are stored at storage-house temperatures (10° to 15°).

At the inception of the present investigations, little information was available regarding the factors influencing the infection of sweetpotatoes by *Diplodia tubericola* and *Sclerotium bataticola*. *D. tubericola* (6) had been observed to infect sweetpotatoes through wounds, more readily in an open than in a closed vessel, to cause infection at temperatures of 12.2° to 13.5° and 34° to 35° C. more readily at the higher than at the lower temperature, and to completely decay roots in 4 to 6 weeks. *S. bataticola* (6) had been observed to infect sweetpotatoes through wounds and to completely decay roots in 3 to 6 weeks.

⁵ To rectify any misconception that may have arisen as a result of statements in the introduction and in footnote 7 of the paper here cited (1), Artschwager has submitted the following: "The impression given by us, through the omission of a corrected statement, that our work consisted of more than a histological study of the materials furnished by J. I. Lauritzen is hereby corrected."

MATERIALS AND EQUIPMENT

The Yellow Jersey (Improved) (9) variety of sweetpotato was used in most of the experiments and the Porto Rico and Nancy Hall varieties were used in a few. The roots of all three varieties were grown and stored at the Arlington Experiment Farm, Rosslyn, Va.

In the experiments dealing with rhizopus soft rot, surface rot, and fusarium end rot, the sweetpotatoes were not inoculated and infection depended upon the pathogenes present on the roots. In the Java black rot and charcoal rot experiments, as well as in the temperature-growth experiments, *Diplodia tubericola* and *Sclerotium bataticola* isolated from sweetpotatoes were used.

The following storage space was employed in these experiments: Infection chambers 3 by 3 by 2 feet high (11), insulated rooms 8 feet in each dimension, and insulated cold-storage rooms 8 by 14 by 11 feet high.

The desired temperatures were obtained by heating (with electric heating coils) either the normal air or air cooled by refrigeration, and the temperature was controlled by electric thermoregulators. The relative humidity was controlled by the use of calcium chloride or water in evaporating pans, and by commercial humidifiers in the case of two of the large rooms when a high humidity was desired.

RHIZOPUS SOFT ROT

INFLUENCE OF TEMPERATURE AND HUMIDITY ON INFECTION

INFLUENCE OF HUMIDITY ON INFECTION AT 23° C.

In an earlier study (12) on the relation of humidity to infection of sweetpotatoes (Yellow Jersey) by *Rhizopus* at a temperature of 23° C., it was found that as the relative humidity fell from a range of 93 to 99 percent to a range of 76 to 84 percent the percentage of infection increased from a range of 10 to 21 to a range of 88 to 100 and decreased as the humidity continued to fall. At relative humidities ranging from 48 to 53 percent the percentage of infection ranged from 23 to 25 percent. It was found that a protective layer of tissue was formed over wounds in the uninfected roots exposed to the higher humidities that limited infection when these roots were stored at any of the humidities from 48 to 95 percent, while most of the uninfected roots from the lower humidities became infected when stored at humidities of 84 to 95 percent. In the suberization-periderm studies of Artschwager and Starrett (1), at a temperature of 23° relative humidities of 49, 65, 76, 87, and 95 percent were employed. The duration of storage at these humidities was 10 days. Suberization occurred at the 3 highest humidities only. A periderm was formed at the 2 higher humidities but not at the 3 lower. It will be seen, therefore, that periderm formation takes place within the range of humidities at which the infection barrier was developed in the Lauritzen-Harter experiments (12) and without doubt is the barrier involved. (The humidities employed in the suberization-periderm experiments (1) were too limited to determine exactly the range of humidities at which these processes occur.) Data confirming this conclusion were obtained in connection with the suberization-periderm experiment (table 1)⁶ of the present investigation.

⁶ The roots in this experiment were halved longitudinally in the same fashion as in the earlier experiment (12), and were stored without inoculation.

TABLE 1.—Influence of humidity at a temperature of 23° C. on suberization and periderm formation and on the infection by *Rhizopus* of sweetpotatoes¹ during 10 days' storage

[19 roots used at each humidity]

Temperature (° C.)	Relative humidity	Roots infected		Suberization ²	Periderm formation ²
		Number	Percent		
23.0	95	3	18	+	+
23.0	87	3	16	+	+
23.6	76	19	100	+	+
22.8	65	0	0	—	—
23.0	49	0	0	—	—

¹ The sweetpotatoes (Yellow Jersey Improved) were harvested Oct. 6 and 7, 1927, cured for 10 days at temperatures of 26° to 29° C., and stored at 10° to 15° and a relative humidity of 90 percent from Oct. 17 to Mar. 3, 1928.

² Plus or minus signs indicate presence or absence of suberization and periderm formation.

Although suberization occurred within 4 days at a relative humidity of 76 percent, it did not prevent infection. Infection was limited at relative humidities above and absent at relative humidities below 76 percent.

The fact (12) that uninfected halved roots first stored at relative humidities below 76 percent for a period became infected when stored for another period at relative humidities above 84 percent (23° C.) indicates that the limitation of infection during the initial storage was at least partly due to the effect of humidity on the pathogenes.

The foregoing data indicate: (1) That relative humidity above 76 percent at 23° C. is a factor favoring the development of suberization and periderm formation and limiting infection, (2) that suberization either is not an effective barrier in preventing infection at a relative humidity of 76 percent at 23° or it does not occur in time to exclude the initiation of decay, and (3) that a periderm is an effective barrier against infection under these conditions.

INFLUENCE OF HUMIDITY ON INFECTION AT 12° C.⁷

It was stated in an earlier report (12) that the results of one experiment, involving a study of the relation of humidity to infection of sweetpotatoes by *Rhizopus* at a temperature of 12° C., "correspond with those obtained at 23°, except that the range of infection was shifted nearer 100 percent humidity." In the light of the results of later and more comprehensive experiments this statement requires qualification, as the apparent resistance to infection in halved roots at high humidities at 12° is not as definite and permanent as at 23° and will yield greater infection during subsequent storage, especially at certain relative humidities.

Nine⁸ experiments were conducted at different periods of the storage season during 3 years. The roots (Yellow Jersey Improved) were halved longitudinally with a sharp knife and stored at the various relative humidities (4 in each experiment) at 12° C. without inoculation. After preliminary storage of 9 to 11 days at the different relative humidities at 12° C. the roots were subjected to another period

⁷ *Rhizopus nigricans* (11) is the pathogene that causes rhizopus soft rot at a temperature of 12° C.

⁸ The roots used in 2 of the experiments were uncured, those used in the remaining experiments were cured for 10 days at a temperature ranging from 25° to 30° C.

of storage (11 to 34 days) at various humidities, at temperatures ranging from 12° to 15.5°. In 6 out of the 9 experiments the percentage of infection was less during the 10 days' preliminary storage at relative humidities ranging from 91 to 98 percent than at those ranging from 62 to 86 percent, with two exceptions (at a relative humidity of 62 percent in one experiment and 85 percent in the other). By the end of the subsequent storage period the percentage of infection was generally less in roots initially stored at relative humidities of 93 to 98 percent than in roots initially stored at the lower humidities. The percentage of infection was always less when the subsequent storage humidity was above 90 percent.

The association of an inhibition of infection of sweetpotatoes by *Rhizopus nigricans* with the formation of a suberin layer at relative humidities of 94 to 98 percent indicates that suberization is a factor limiting infection; however, it is not so effective a barrier as is a periderm. If periderm formation played a part in inhibiting infection at these humidities, it was only during the latter part of the storage period.

The failure of a suberin or periderm layer to form on wounded surfaces at the lower humidities explains the heavy infection in roots exposed under these conditions. If the relative humidity becomes low enough to limit infection, the inhibition is removed as soon as the roots exposed to such humidities are stored at the higher humidities (above 90 percent). This fact will become more evident from the results to be discussed presently.

In roots initially stored at a relative humidity of 93 to 97 percent at 12° C., followed by subsequent storage at 12° to 15° and various relative humidities, the percentage of infection was lower in the experiments started in the early part of the season (before Dec. 22) than in those started later. Earlier results (11) show that *Rhizopus* generally is less abundant on the roots early in the storage season than later and that freshly dug potatoes become infected as readily as old ones when they are inoculated by dipping in a water spore suspension.

INFLUENCE OF HIGH HUMIDITIES ON INFECTION AT 12° TO 36.5° C. AND OF DIFFERENT HUMIDITIES AT 12°

Data were obtained from two experiments in which longitudinally halved, cured and uncured roots (Yellow Jersey) were initially and subsequently stored as indicated in table 2.

With the exception of 2 percent infection at 31° C. during the initial storage period, there was no infection by *Rhizopus* at any of the temperatures from 22° to 31° during either the initial or the subsequent storage periods. The results at the various humidities at 12° are similar to those already discussed. It should be noted that when storage was continuous at relative humidities of 96 and 97 percent the infection remained relatively small (5 and 17 percent) throughout the experiment, whereas in roots initially stored for 11 days at 96 to 97 percent relative humidity and subsequently for 20 days at relative humidities of 80 and 83 percent the percentage of infection was rather high (80 and 40 at 83 and 80 percent relative humidity, respectively). The percentage of infection, however, was not so high as in roots originally stored at relative humidities of 82 to 83, 73 to 80, and 60 to 67 percent, in which it reached 100 percent by the end of 10 days' final storage at 96 to 97 percent relative humidity.

TABLE 2.—Influence of temperature and relative humidity on infection of sweet-potatoes by *Rhizopus*¹

Initial 11 days' storage						Subsequent storage ¹					
Storage conditions				Roots used (total for the 2 experi- ments) ¹	Roots infected (total for the 2 experi- ments) ¹	Storage conditions		Roots used	Total infection after—		
Experiment 1		Experiment 2				Tem- per- ature	Rela- tive hu- midity		10 days	20 days	
Temperature (° C)	Rela- tive hu- midity	Tem- per- ature	Rela- tive hu- midity								
	Percent	° C	Percent	Number	Percent	° C	Percent	Num- ber ²	Percent	Percent	
31	92	31	93	59	2	31	92	58	0	0	
24	93	24	94	60	0	24	93	60	0	0	
22.5	93	22	94	59	0	22.5	93	59	0	0	
22	95	21.5	94	59	0	22	95	59	0	0	
15.5	92	15.5	95	60	3	15.5	92	59	3	5	
12	96	12	97	62	6	12	96-97	38	5	17	
12	82	12	83	60	35	12	96-97	39	100	—	
12	73	12	80	60	47	12	96-97	32	100	—	
12	60	12	67	60	8	12	96-97	55	100	—	
12		12	97			12	83	10	50	80	
		12	97			12	80	10	20	40	

¹ With the exception indicated in footnote 3, the results given in this table are totals of 2 experiments. The sweetpotatoes were harvested Oct. 11, 1928. Experiment 1 was started immediately with uncurd roots, experiment 2 was started Oct. 23, after 12 days' curing of the roots at 21° to 28° C.

² The numbers in this column at temperatures from 15.5° to 31° that do not correspond to the original numbers are instances in which the roots were removed from the experiment at the end of the preliminary storage period.

³ Two lots of the roots originally stored at 12° C. and 97 percent relative humidity were stored subsequently at relative humidities of 80 and 83 percent, respectively.

There was some *Rhizopus* infection in roots stored continuously at a temperature of 15.5° C. It would seem that at this temperature there was a race between wound-periderm formation and infection. While Artschwager and Starrett (1) found periderm formation in one instance at 14.7° and 94 percent relative humidity in 11 days, it was not found in another case at 15.7° and 92 percent relative humidity in 10 days.

Another experiment was run (table 3) in which halved roots were first stored for a period of 10 days at a range of temperatures from 12° to 36.5° C. combined with high humidities at each temperature and a range of four humidities (from 68 to 96 percent) at 12°. After this period of initial storage, four lots of the uninfected roots from each of these combinations of temperature and humidity were stored, respectively, at relative humidities of 95, 82, 78, and 68 percent at 12° for ensuing periods of 10 and 20 days.

By the end of the preliminary storage period, 5 percent of the roots stored at 36.5° C. and a relative humidity of 96 percent and 2 percent of those stored at 22° and a relative humidity of 95 percent had become infected by *Rhizopus* (table 3). There was no infection between 22° and 36.5°, during either the initial or the subsequent storage periods. There was infection at 15.5° during the initial storage and by the end of 20 days of final storage at the three lowest humidities at 12°.

Some resistance to infection was built up in the roots stored originally at 12° C. and a relative humidity of 96 percent and it was maintained more strongly when the storage was continued at that temperature with approximately the same relative humidity than with lower humidities. All the roots originally stored at the 3 lowest humidities became infected after a period of further storage at 12°

TABLE 3.—*Influence of temperature and relative humidity on infection of cured sweetpotatoes by Rhizopus, Nov. 15 to Dec. 15, 1928*

Initial 10 days' storage			Subsequent storage											
Tem- pera- ture	Rela- tive humid- ity	Roots used	Infection in 10 days at 12° C. and relative humidity indicated						Infection in 20 days at 12° C. and relative humidity indicated					
			95 percent		82 percent		78 percent		68 percent		95 percent		82 percent	
			Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected
° C.	Percent	Number	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
36.5	96	60	15	0	14	0	14	0	15	0	14	0	14	0
31.5	90	60	15	0	15	0	15	0	15	0	15	0	15	0
24.5	94	60	15	0	15	0	15	0	15	0	15	0	15	0
22	95	60	15	0	15	0	14	0	15	0	15	0	14	0
22	93	60	15	0	15	0	15	0	15	0	15	0	15	0
15.5	95	60	15	0	15	0	15	0	15	0	15	0	15	0
12	96	60	13	23	13	69	14	7	14	8	13	38	14	43
12	81	60	87	2	100	2	100	46	12	50	2	50	13	58
12	78	60	63	6	100	6	53	2	5	20	6	50	5	20
12	68	60	0	7	15	7	40	40	15	0	15	53	15	47

and 95 percent relative humidity. Some of the roots from each of these 3 relative humidities remained uninfected when stored at a relative humidity of 68 percent, some of those originally stored at the 2 lowest humidities remained uninfected when stored at a relative humidity of 78 percent, and some of those originally stored at the 2 lowest humidities remained uninfected during the final storage at the 3 lowest humidities. More of the roots originally stored at 81 percent relative humidity became infected during the entire period of storage than roots from any of the other initial humidities. In this experiment temperature and humidity as they affect the host and humidity as it affects the pathogene become factors in the infection and decay of sweetpotatoes by *Rhizopus*. When temperature and humidity were such as to favor suberization and periderm formation (12° to 36.5° combined with high humidities), infection was limited because of the difficulty or inability of the pathogene to penetrate the suberized or periderm layers.

Infection at relative humidities of 68 and 78 percent at 12° C. was less during the initial storage period than at 81 percent relative humidity and remained less during subsequent storage periods at relative humidities of 68 and 78 percent. In the absence of suberization and periderm formation to inhibit infection at humidities of 68 and 78 percent, it would seem that it was the effect of humidity on the pathogene that limited infection during the initial storage, because when more moisture (at 95 percent relative humidity) was added to the environment the pathogene (*Rhizopus nigricans*) was able to initiate infection and decay.

INFLUENCE OF HUMIDITY ON INFECTION AT 28° TO 29° C.

Seven experiments were run during three storage seasons (1928-29, 1929-30, and 1930-31) in which roots halved longitudinally were stored at 5 relative humidities in each experiment (except one in which there were only 4 relative humidities) at temperatures of 28° to 29° C. for a period of 10 days, and subsequently at temperatures from 10° to 16° at various relative humidities for another period of 19 to 31 days (table 4).

The results differed somewhat in the various experiments, for which reason they are submitted in detail. The factors that may have played a part in producing this variation were (1) the amount and uniformity of inoculum present, (2) the number of roots used, and (3) the variation of the subsequent storage.

The relation of relative humidity to infection was less definite in experiments run early in the season (experiments 2, 3, and 4) than in those run later, with the exception of experiment 5. In this latter case the number of roots used was too small to give an accurate picture of the effect of humidity on infection. The low relative humidity of the subsequent storage in experiments 4 and 5 may have played a part in producing the rather large percentage of infection in roots initially stored at a relative humidity of 96 percent. It has been observed that halved roots, healed over at high humidities, sometimes crack open in the wounded areas when they are subjected to storage at low humidities. Such wounds may give rise to new infections. If the results of the seven experiments are assembled and averaged in accordance with the initial storage humidities (table 5) it will be seen that there was a fairly definite relation between the rel-

ative humidity and the percentage of infection. Barring certain exceptions, the percentage of infection tended to increase with the lowering of the relative humidity. These results indicate that infection is inhibited over a fairly wide range of humidities at a temperature of 28° to 29° C. as a result of wound healing and that the percentage of infection is influenced by the rate of healing.

TABLE 4.—*Influence of relative humidity on infection of sweetpotatoes by Rhizopus at 28° to 29° C.¹*

Experiment no	Dates	Initial 10 days' storage				Subsequent storage			
		Temperature	Relative humidity	Roots used	Roots infected	Temperature	Relative humidity	Duration	Roots infected
		° C	Percent	Number	Percent	° C	Percent	Days	Percent
1	Feb 12 Mar 13, 1929	29	95	20	0	10	84	19	0
		29	91	20	0	10	84	19	0
		29	83	20	5	10	84	19	5
		29	74	18	0	10	84	19	11
		29	66	19	0	10	84	19	26
2	Oct. 11–Nov 21, 1929	28	96	10	0	15.5	87	31	0
		28	90	10	0	15.5	87	31	0
		28	78	10	20	15.5	87	31	20
		28	69	10	0	15.5	87	31	0
		28	59	10	10	15.5	87	31	10
3	Oct 11–Nov. 14, 1930	28	98	40	0	15.5	84	24	0
		28	84	40	2 5	15.5	84	24	55
		28	74	40	0	15.5	84	24	0
		28	72	40	0	15.5	84	24	40
		29	96	10	0	15.5	58	31	50
4	Oct 25 Dec 5, 1929	29	90	10	20	15.5	58	31	20
		29	87	10	0	15.5	58	31	0
		29	71	10	20	15.5	58	31	20
		29	63	10	0	15.5	58	31	0
		29	96	6	33	13	50	22	50
5	Jan 23 Feb 24, 1930	28	89	6	17	13	50	22	17
		28	78	6	17	13	50	22	17
		28	69	6	17	13	50	22	17
		28	62	6	17	13	50	22	17
		29	96	80	2 5	15.5	92	21	2 5
6	Jun 21 Feb 21, 1931	28	88	80	5 0	15.5	92	21	6 0
		28	78	80	34.0	15.5	92	21	34 0
		28	72	80	54 0	15.5	92	21	66.0
		29	62	80	66 0	15.5	92	21	75 0
		28	96	80	36	16	92	20	36
7	Feb 3 Mar 5, 1931	28	89	80	20	16	92	20	21
		28	77	80	48	16	92	20	50
		28	72	80	64	16	92	20	73
		29	62	80	53	16	92	20	60

¹ Uncured roots were used in experiments 2 and 3, and cured roots in the others.

TABLE 5.—*Influence of relative humidity on infection of sweetpotatoes by Rhizopus at 28° to 29° C., as based on data in table 4*

Relative humidity	Roots used	Roots infected			
		During initial storage		During initial and final storage	
		Number	Percent	Number	Percent
Percent	Number				
95-98	246	33	13	39	16
88-91	207	23	11	25	12
83-87	70	2	3	23	33
74-78	234	68	29	72	31
69-72	226	97	43	130	58
59-66	205	97	47	115	56

INFLUENCE OF TYPES OF WOUNDING ON INFECTION BY RHIZOPUS AT DIFFERENT TEMPERATURES AND HUMIDITIES

During the seasons 1928-29 and 1929-30, four experiments were conducted in which sweetpotatoes (Yellow Jersey) were subjected to 10 different types of wounding and stored without inoculation at 15 combinations of temperature and relative humidity (tables 6 and 7)

for a period of 10 days and then stored at a common temperature and relative humidity for another period. The temperatures and relative humidities of the subsequent storage period were as follows: Experiment 1, 10° C. and 84 percent relative humidity; experiment 2, 15.5° and 87 percent; experiment 3, 15.5° and 58 percent; and experiment 4, 13° and 67 percent. The duration of the subsequent storage was 19, 31, 31, and 21 days, respectively. The number of roots stored at each initial combination of temperature and humidity in each experiment was from 5 to 20, but in most cases 10. In three of the experiments the roots were cured and in one they were not.

TABLE 6.—*Influence of 10 types of wounding on infection of sweetpotatoes by Rhizopus at different temperatures and humidities during 10 days' storage*¹

Type of wounding ²	Percentage ³ of roots infected at indicated temperatures (°C., above) and relative humidities (percent, below)							
	35-37 91-95	31-32 88-95	28-29 95-96	25 91-94	21-22 91-94	10-21 89-93	15-16 89-95	12 93-96
Bruised tip	8.6	11.4	8.0	11.4	46.0	35.3	37.1	40.0
Bruised side	19.4	5.7	4.0	23.0	42.9	40.0	28.6	45.7
Broken tip	2.8	0	4.0	0	0	0	0	0
Cross section tip	0	0	0	0	0	2.9	0	0
Cross section medium	0	0	0	0	0	0	2.9	0
Cross section half	0	0	6.7	0	0	0	2.5	5.0
Longitudinal section shallow	2.9	0	0	0	2.9	0	0	0
Longitudinal section medium	0	0	0	0	0	0	0	0
Longitudinal section half	4.0	0	0	16.0	0	0	24.0	13.7
Skinned	0	0	0	0	0	0	0	0

Type of wounding ²	Percentage ³ of roots infected at indicated temperatures (°C., above) and relative humidities (percent, below)								
	24-29 95-96	28-29 90-91	28-29 78-83	28-29 69-74	28-29 59-66	12 93-98	12 84-86	12 70-77	12 62-65
Bruised tip	8.0	0.0	0.0	0.0	0.0	40.0	5.7	0.0	2.9
Bruised side	4.0	8.3	24.0	0	4.0	45.7	51.4	45.7	20.0
Broken tip	4.0	0	4.0	0	0	0	0	0	0
Cross section tip	0	4.2	0	0	0	0	0	0	0
Cross section medium	0	0	0	0	0	0	5.7	0	0
Cross section half	6.7	0	0	6.3	0	5.0	20.0	15.0	2.5
Longitudinal section shallow	0	0	0	0	0	0	2.9	0	0
Longitudinal section medium	0	0	0	0	0	0	14.3	5.0	0
Longitudinal section half	0	4.0	7.5	5.1	2.6	13.7	24.0	34.0	26.5
Skinned	0	0	0	0	0	0	0	0	0

¹ The results given in this table were obtained from 4 experiments in each of which from 5 to 20 roots (10 in the majority of cases) were used for each type of wounding and for each combination of temperature and humidity. The experiments were started Dec. 22, 1928, and Feb. 12, Oct. 11, and Oct. 25, 1929. Cured roots were used in 3 experiments and uncured roots in 1.

² For definitions of types of wounding see text below.

³ The percentages are based on from 35 to 50 roots

A description of the various types of wounding follows. (1) One end of the roots was tapped on a laboratory desk, bruising the tissue from 5 to 10 mm deep (bruised tip). (2) The roots at their thickest diameter were struck on the rim of a wire basket, thus bruising the root tissue to a depth of about 5 to 8 mm (bruised side). (3) One tip end of the roots was broken off with the fingers at a point where the root was about 7 to 10 mm in diameter (broken tip). (4) One tip end was cut off with a sharp knife back from the tip where the root was about 7 to 10 mm in diameter (cross section tip). (5) The roots were cut in cross section about midway between the one tip end and the thickest diam-

eter of the root, and the larger portion was stored (cross section medium). (6) The roots were halved with a sharp knife in cross section (cross section half). (7) A small slice, about 1 to 1.5 cm in diameter, was cut from the side of the root between the skin and the outer vascular tissue (longitudinal section shallow). (8) A slice was taken from the side of the root to a depth of about one-fourth of its diameter (longitudinal section medium). (9) The roots were halved with a sharp knife longitudinally (longitudinal section half). (10) The roots were skinned in 2 to 3 places (the number of skinned areas on a root was the same in a given experiment) in areas from 8 to 10 mm in diameter (skinned).

TABLE 7.—*Influence of further storage at 10° to 15.5° C. and relative humidities of 53 to 87 percent on infection by Rhizopus of the roots discussed in connection with table 6¹*

Type of wounding	Percentage ² of roots infected as related to indicated initial temperatures (°C., above) and relative humidities (percent, below)							
	35-37 91-95	31-32 88-95	28-29 95-96	25 91-94	21-22 91-94	19-21 89-93	15-16 89-95	12 93-98
Bruised tip	8.6	11.4	8.0	11.4	46.0	35.3	40.0	45.7
Bruised side	19.4	5.7	4.0	23.0	42.9	40.0	28.6	45.7
Broken tip	8.3	0	4.0	0	0	0	8.6	2.9
Cross section tip	0	0	0	0	0	2.9	0	0
Cross section medium	0	0	0	0	0	0	5.7	8.6
Cross section half	2.5	0	10.0	0	0	0	5.0	20.0
Longitudinal section shallow	2.9	0	0	0	2.9	0	0	0
Longitudinal section medium	5.5	0	0	0	2.7	0	5.7	8.6
Longitudinal section half	6.0	0	12.5	18.0	2.0	2.0	46.0	56.9
Skinned	3.3	0	0	0	0	0	0	0

Type of wounding	Percentage ² of roots infected as related to indicated initial temperatures (°C., above) and relative humidities (percent, below)								
	28-29 95-96	28-29 90-91	28-29 78-83	28-29 69-74	28-29 59-66	12 93-98	12 84-86	12 70-77	12 62-65
Bruised tip	8.0	8.3	0.0	0.0	0.0	45.7	14.3	0.0	2.9
Bruised side	4.0	8.3	24.0	0	4.0	45.7	54.3	54.3	40.0
Broken tip	4.0	0	4.0	0	0	2.9	8.6	0	2.9
Cross section tip	0	4.2	0	0	0	0	0	0	0
Cross section medium	0	0	0	0	4.0	8.6	25.7	34.3	28.9
Cross section half	10.0	0	0	9.4	6.7	20.0	47.5	67.5	47.5
Longitudinal section shallow	0	0	0	0	0	0	11.4	16.7	2.9
Longitudinal section medium	0	0	0	0	4.0	8.6	34.3	47.5	28.0
Longitudinal section half	12.5	4.9	7.5	10.3	15.4	56.9	72.0	98.0	84.0
Skinned	0	0	0	0	0	0	10.0	3.3	0

¹ The results given in this table were obtained from the 4 experiments discussed in connection with table 7. The duration of the storage subsequent to the initial 10 days' storage was 19, 31, 31, and 21 days, respectively. Values are total percentages of infection for initial and subsequent storage periods combined.

² Based on 35 to 80 roots.

The results from the four experiments on the presence or absence of infection were so similar that it was decided to combine them in order to simplify the presentation of the data. There was some variation in the percentage of infection under given conditions in a given experiment but not sufficient to alter the general relations found. Table 6 shows the percentages of roots infected in the four experiments at the end of the initial period of storage, and table 7 shows the percentages of infection for the initial and subsequent storage periods combined.

The data of tables 6 and 7 reveal marked differences in the percentage of infection as correlated with the type of wounding. During the

initial storage period at the various temperatures (combined with high humidities) infection was more consistently present in roots bruised at the tip and in those bruised at the side. By the end of the ensuing storage period the same was true in roots bruised at the tip, in those bruised at the side, and in those halved longitudinally. Infection was more uniformly present in roots with bruised tips and in roots with bruised sides than in roots halved longitudinally. Bruising interferes with healing (13) more than do the other types of wounding, and hence favors heavier infection. The higher percentage of infection in halved roots than in the other types of wounding, except bruising, is believed to be due to the greater area of wounded tissue. No infection by *Rhizopus* occurred on the skinned roots during the initial period of storage and very little during the ensuing period. There was either no infection or the percentage was relatively small on roots cut in cross section at the tip or broken tip either during the preliminary or subsequent storage. In general, the greater the extent of wounded area in cut roots the greater the percentage of infection. There was very little change in the percentage of infection following the initial storage in roots bruised at tip or side and originally stored at temperatures from 12° to 35° to 37° C. combined with high humidity. In the case of roots bruised at tip or side the percentage of infection was higher at temperatures ranging from 12° to 22° (combined with high humidity) than at temperatures above 22° at the end both of initial and of final storage. The initial storage at temperatures of 12° to 16° and high humidities generally yielded more infection (table 7) by the end of the final storage period in some of the types of wounding (cross section medium, cross section half, longitudinal section medium, and longitudinal section half) than did the initial storage at temperatures from 19° to 37°. Although generally there was little infection at any of the temperatures and humidities during either the initial or ensuing storage periods, in roots subjected to types of wounding designated as broken tip, cross-section tip, and longitudinal section shallow, it will be seen later that temperatures from 12° to 16° were critical in these roots as well as in roots subjected to the other types of wounding.

These results show that temperature combined with high humidity limited infection more in cut surfaces than in bruised tissue. In the former case cork formation was free to proceed at its normal rate, and above 15° to 16° C. this was sufficiently rapid to intercept most of the infection. At 16° and below, the rate of cork formation was retarded enough to permit of infection (in roots with the larger cut areas), which generally increased in percentage (tables 6 and 7) with the lowering of the temperature. The infection rate and the cork-formation rate curves cross somewhere between temperatures of 15° to 16° and 19° to 21°. In bruised tissue healing was inhibited and infection took place more or less readily at all the temperatures. The bruised tissue, in addition to interfering with healing, forms an excellent matrix for the pathogenes (*Rhizopus nigricans* and *R. tritici*) to establish a foothold in the host tissue. The fact that temperatures above 22° and at 28° to 29°, irrespective of humidity, tend to limit infection indicates the possibility that some suberization and periderm formation occur in bruised tissue at these temperatures. A periderm⁹ has been observed to occur in bruised tissue at temperatures of 25° and 29°.

⁹ Observations made by the writer and L. L. Harter in connection with a study of the effect of temperatures from 25° to 29° C. in checking decay of sweetpotatoes by *Rhizopus nigricans*.

In these experiments (tables 6 and 7) there was little relation between infection and relative humidity at 28° and 29° C., except possibly in roots halved longitudinally, by the end of the final storage period. Barring the rather high percentage (12.5 percent) of infection at relative humidities of 95 to 96 percent, there was an increase in the percentage of infection with lowering of the relative humidity. Whether the exception was accidental and associated with insufficient numbers to iron out individual variations or whether such high humidities at 28° to 29° are peculiarly favorable to the activities of the pathogenes is not known. Such exceptions have occurred in a number of experiments, but in others there has been no infection at all at this humidity. This tendency for the percentage to increase with the lowering of the relative humidity is correlated with the increase in time required for cork formation to take place with the lowering of the relative humidity. It would seem that the chances of infection are greater and those for complete healing less in longitudinally halved roots than in the other cut surfaces because of the greater wounded area of the former. The chances for complete healing might well decrease to a greater extent in the larger than in the smaller wounds with the lowering of the humidity. Infections would then be expected to increase with lowering of humidity provided the humidity of the ensuing storage did not too greatly inhibit the activities of the pathogenes. When the cut surfaces are limited, especially in cross section tip and cross section medium, and in longitudinal sections shallow and medium (tables 6 and 7), it appears that temperatures of 28° to 29° are sufficiently favorable to healing to more than counterbalance the retarding effect of an unfavorable humidity.

Artschwager and Starrett (1) obtained suberization at 28° C. at relative humidities of 73.5, 82.7, 90.6, and 95 percent within 48 hours and periderm formation at relative humidities of 90.6 and 95 percent within 96 hours, at 82.7 percent within 120 hours, and at 73.5 percent within 144 hours. At 66 percent relative humidity, suberization occurred in 6 days but no periderm was formed within 11 days. This humidity, as well as that of 73.5 percent, probably has some effect on the pathogenes inhibiting infection during the initial storage period, and since suberization occurred by the end of 6 days it is probable that its formation and a possible later formation of periderm may have limited infection during the subsequent storage period.

In roots stored at the various relative humidities at 12° C. the different types of wounding had a marked influence on the amount of infection. During the initial storage period rather heavy infection occurred in roots that had been bruised at tip, bruised at side, or halved in cross section or longitudinally. During the ensuing storage period the percentage of infection increased quite definitely with the extent of wounding in cut surfaces, no infection occurring in the cross-section-tip type and the percentage remaining comparatively small in the longitudinal-shallow type. The percentage of infection was also relatively small in skinned roots and in roots with broken tips. In the types of wounds, aside from roots bruised at tip and cross section tip (table 7), the two extremes of relative humidity at 12° C. (93 to 98 and 62 to 65 percent) generally limited infection more during the final storage period than did the adjacent intermediate humidities (84 to 86 and 70 to 76 percent). This

tendency was also present during the initial storage (table 6) whenever infection occurred. The factor limiting infection at relative humidities from 93 to 98 percent was probably suberization and possibly periderm formation during the latter part of the final storage period. At 62 to 65 percent humidity there was a horny layer formed over a cut surface that tended to limit infection unless the roots were subsequently stored at high humidity (above 90 percent). The relative humidities employed during the subsequent storage were 58, 67, 84, and 87 percent.

CONCLUSIONS

The following conclusions may be drawn in regard to the effects of temperature, humidity, types of wounding, suberization, and periderm formation on infection of sweetpotatoes by *Rhizopus*. (1) Since 12° C. is near the lower temperature limit for suberization and periderm formation and since these processes apparently occur only at relative humidities between 90 and 100 percent (10 and 11 days were required for suberization to take place at 12° and a relative humidity of 94 percent and 7 days at 96 percent, and the shortest period at which periderm formation was observed to occur at this temperature was 25 days), infection by *Rhizopus* takes place readily at this temperature. (2) Since suberization and periderm formation take place more rapidly as the temperature is raised above 12° to at least 32°, infection by *Rhizopus* tends to be limited with the rise in temperature within the limits specified. It has been shown by earlier work (11) that as the temperature is raised above 33° infection takes place more readily and occurs even in unwounded roots. The factors involved in this increased infection have not been determined. Whether the slowing up of the healing processes at temperatures above 32° is a factor in increased infection is not known. (3) Humidity is more of a limiting factor in infection at 23° than at 28° and 29° because of its effect on suberization and periderm formation. (4) Bruising, because of its effect on suberization and periderm formation, permits of infection fairly readily at most of the temperatures (within the limits of those discussed) and at most humidities except at 28° to 29°, where there was some irregularity of infection at the various relative humidities. (5) Infection is correlated more or less with the extent and degree of wounding. (6) While low humidities at temperatures of 12° and 23° inhibit infection in freshly wounded roots because of their effect on the pathogenes, infection takes place readily when the roots are returned to humidities above 90 percent.

SHRIVELING, SUPERFICIAL GROWTH OF *PENICILLIUM*, AND INVASION OF WOUNDED AREAS OF SWEETPOTATOES BY CERTAIN FUNGI AS RELATED TO HEALING, TEMPERATURE, AND HUMIDITY

In connection with the *Rhizopus* studies, observations were made on the effect of 10 days' initial storage of wounded sweetpotatoes at different temperatures and relative humidities on shriveling, superficial growth of *Penicillium*, and invasion of wounded areas by certain fungi during 47 days of subsequent storage at 10° C. and 84 percent relative humidity. The observations were made on the following types of wounding: Longitudinal shallow, longitudinal medium, longitudinal half, cross section medium, and cross section half (table 8).

TABLE 8.—*Shriveling, superficial growth of Penicillium, and invasion by certain fungi in wounded sweetpotatoes initially stored for 10 days at different temperatures and relative humidities and subsequently for 47 days at 10° C. and 84 percent relative humidity*

Initial storage temperature (° C.)	Relative humidity	Data on roots wounded as indicated									
		Longitudinal shallow					Longitudinal medium				
		Roots showing Penicillium on cut surface	Roots showing cut areas sunken	Depth of sunken areas	Roots invaded	Average depth of decay	Roots used	Roots showing Penicillium on cut surface	Roots showing cut areas shriveled	Degree of shriveling	Roots invaded
		Number	Number	Mm	Number	Mm	Number	Number	Number	Number	Number
35	91	10	0	0	0	0.0	10	0	0	Slight	0
32	90	10	0	0	0	0	10	0	2	do	0
29	95	10	0	0	0	0	10	1	0	do	0
26	92	10	0	0	0	0	10	0	0	None	0
25	94	10	1	2	1	3.0	9	0	0	do	0
22	93	10	1	0.5-1	1	3.0	10	0	2	Very slight	0
19	94	10	3	0.5-3	9	1.2	10	7	8	Marked	8
15	94	10	8	1-3	10	1.3	10	1	10	do	9
12	95	10	0	5	0	0	10	1	2	Slight	0
29	91	10	0	5	0	0	10	1	0	do	0
29	83	10	3	5-1	0	0	10	1	6	do	2
29	74	10	3	5-1	0	0	10	5	6	Slight to marked	3
29	66	10	8	5-1.5	0	0	10	6	10	do	7
22	94	10	10	1-3	10	1.3	10	7	10	Marked	8
12	84	10	9	2-4	10	2.5	8	8	4	Very marked	4
12	77	9	9	2-5	8	2.8	4	4	4	do	4
12	62	10	10	2-6	10	3.0	3	3	3	do	3
		Number	Number	Mm	Number	Mm	Number	Number	Number	Degree of shriveling	Number
		Very bad.									
		Slight									
		Do									
		Do									
		Slight to marked.									
		Marked.									
		Bad.									
		Slight									
		Marked.									
		Do									

¹ Where there are no data recorded, the roots not infected by *Rhizopus* were discarded before these data were taken

TABLE 8.—Shriveling, superficial growth of *Penicillium* and invasion by certain fungi in wounded sweetpotatoes initially stored for 10 days at different temperatures and relative humidities and subsequently for 47 days at 10° C. and 84 percent relative humidity.—Continued

Initial storage		Data on roots wounded as indicated											
		Cross section medium					Cross section half						
Temperature (°C.)	Relative humid- ity	Roots used	Roots showing Penicil- ium on cut sur- face	Roots showing cut areas shriveled	Degree of shriveling	Roots in- vaded	Average depth of decay	Roots used	Roots showing Penicil- ium on cut sur- face	Roots showing shriveling	Degree of shriveling	Roots in- vaded	Average depth of decay
Per- cent		Number	Number	Number		Number	Mm	Number	Number	Number		Number	Mm
35	91	10	0	1	Marked	1		10	0	7	Slight to bad	6	7.7
32	90	10	0	0	None	0		10	1	3	do	3	7.7
29	95	10	1	0	Slight	3	1.7	10	0	3	Marked	4	9.5
25	92	10	1	0	None	3	1.0	10	4	3	Marked to very marked	4	13.0
22	94	10	1	2	Slight	2	4.5	10	0	1	Slight	2	1.5
19	93	10	1	0	None	2	2.5	10	0	7	Marked	2	5.7
15	94	9	9	9	Marked	10	6.3	10	9	10	Very marked	10	7.3
12	94	8	8	8	do	8	17.0	9	9	9	do	9	7.7
29	95	10	1	1	Slight to marked	3	1.7	10	0	4	Marked	4	9.5
26	91	10	0	2	do	2	3.0	10	0	5	Slight to marked	4	8.5
23	83	10	2	2	do	0		10	5	9	do	7	3.4
20	74	9	5	5	Slight to marked	4	2.3	9	9	9	Marked to very marked	9	6.8
20	66	9	7	6	do	3	10.0	9	9	9	do	9	16.0
13	84	8	8	8	Marked	8	17.0	9	9	9	Very marked	9	7.4
12	84	9	9	9	do	9	12.0	4	4	4	do	4	17.0
12	77	4	4	4	do	4	4.5	2	2	2	do	2	12.0
12	62	4	4	4	Very marked	4	14.0	3	3	3	Bad	3	14.0

Temperature during the initial storage period was a factor affecting shriveling, largely, it is believed, because of its influence on suberization and periderm formation. At temperatures of 22° C. and above (combined with high humidity) there was less shriveling than at 12°, 15°, and sometimes 19° (combined with high humidity), although the saturation deficit was smaller at these temperatures than at the higher temperatures.

Data taken from the experiments under discussion show that suberization and periderm formation take place in a shorter time at temperatures from 22° to 32° than at 19° C. and below. Considerable evaporation of moisture probably took place at the lower temperatures before suberization and periderm formation occurred. There was a tendency for the number of roots showing shriveling to increase with the lowering of the relative humidity at 29°. This increase in shriveling was correlated with the lowering of the relative humidity and the corresponding increase in time required for suberization and periderm formation. Practically all the roots, irrespective of the type of wounding, showed sunken areas or shriveling¹⁰ at all the relative humidities at 12°.

The presence of *Penicillium* and infection were closely associated with conditions of temperature and relative humidity during initial storage favoring shriveling—conditions that either retarded or did not permit of suberization and periderm formation.

Out of 156 isolations made from infected roots, 47.4 percent yielded *Penicillium*, 21.8 percent *Fusarium*, 5.8 percent unidentified fungi, 3.9 percent *Alternaria*, 1.9 percent *Botrytis*, and 19.2 percent were sterile. Temperature and humidity did not seem to influence the occurrence of any of these fungi directly or independently of their effect on healing and shriveling. These fungi, except perhaps *Fusarium*, normally are not active sweetpotato pathogens but invade wounded tissue left unprotected by lack of suberization and periderm formation.

SURFACE ROT

METHOD OF INFECTION

Infection of sweetpotatoes with surface rot (*Fusarium oxysporum*) takes place chiefly through broken secondary roots, sprouts, and wounds produced by skinning. Wounds deeper than those made by skinning, if not properly healed, usually give rise to other types of decay. Infection through secondary roots generally occurs in freshly dug and improperly cured roots. Infection through sprouts is less frequent and probably occurs only when the sprouts are broken off, injured, or killed in some way.

RELATION OF WOUNDING, TEMPERATURE, AND HUMIDITY TO INFECTION IN CURED AND UNCURED SKINNED SWEETPOTATOES

Four experiments were conducted during 2 seasons (1928–29 and 1929–30) in which sweetpotato roots (Yellow Jersey Improved), skinned in 2 or 3 places (skinned areas about 8 to 10 mm in diameter), were stored at different temperatures and relative humidities for 10 days and subsequently stored at a common temperature and relative humidity for varying periods of time.

The results of all 4 experiments were so similar that the data from only 2 are presented (table 9). In one of these experiments, roots

¹⁰ The degree of shriveling noted was only estimated, except where the depth of the shrinkage is given, and must therefore be regarded as only roughly approximate.

taken directly from the field and skinned without curing were stored at the various initial temperatures and relative humidities; in the other experiment, the roots were cured for 10 days at a temperature of 21° to 24° C. and a relative humidity varying from 52 to 82 percent, and were subsequently stored at a temperature of about 10° to 15° and a relative humidity of about 73 percent for 115 days before skinning and initial storage. The initial and subsequent storage temperatures and relative humidities are given in table 9.

In addition to dealing with the relation of skinning to infection in cured and uncured roots, the results recorded in table 9 contain data on unskinned uncured roots that are discussed later (p. 303).

With the exception of the infection that occurred at a temperature of 25° C. and 91 percent relative humidity, there was no infection in uncured skinned roots initially stored at temperatures from 21° to 36.5° combined with high humidity. The writer has no explanation for the heavy infection at 25°. One infection occurred at this temperature in 1 of the other 3 experiments, but none occurred in the other 2 experiments. There was an increase in the percentage of infection as the temperature was lowered from 21° to 12°.

At a temperature of 28° C. infection occurred at relative humidities of 59, 69, and 78 percent; the lower the humidity the higher was the percentage of infection. No infection occurred at this temperature at relative humidities of 90 and 96. At a temperature of 12° a high degree of infection occurred at all the relative humidities. Although it was lower at relative humidities of 86 and 93 percent than at 65 and 75 percent, this difference involved the absence of infection in only one skinned area at each humidity.

TABLE 9.—Effect of temperature and humidity during 10 days' storage of skinned and unskinned uncured roots and skinned cured roots on the development of surface rot (*Fusarium oxysporum*) during 154 days' subsequent storage at 15.5° C. and 87 percent relative humidity in the case of uncured roots and 58 days' subsequent storage at 10° and 84 percent relative humidity in the case of the cured roots ¹

Uncured roots						Cured roots (skinned)			
Initial storage conditions		Skinned		Unskinned		Initial storage conditions		Skinned areas infected	Depth of decay
Temperature (° C.)	Relative humidity	Roots infected	Skinned areas infected	Roots infected	Average lesions per root	Temperature	Relative humidity		
	Percent	Percent	Percent	Percent	Number	°C	Percent	Percent	Mm
36.5	95	0	0	0	0.0	35	91	0	0 0
31	95	0	0	0	0	32	90	0	0
28	96	0	0	0	0	29	95	0	0
25	91	60	50	0	0	25	92	0	0
21	91	0	0	60	5.1	22	94	0	0
19	89	60	50	44	2.0	19	93	7	1.0-2
16	89	80	80	70	14.5	15	94	77	.5-5
12	93	100	90	100	19.1	12	94	90	.5-4
28	96	0	0	0	0	29	95	0	0
28	90	0	0	10	.8	29	91	0	0
28	78	40	40	30	1.7	29	83	10	.5
28	69	80	70	90	6.1	29	74	6	.5
28	59	100	100	50	4.4				
12	93	100	90	100	19.1	12	94	90	.5-4
12	86	100	90	100	17.4	12	84	87	.5-5
12	75	100	100	100	36.6	12	77	83	.5-5
12	65	100	100	100	30.0	12	66	90	.5-4

¹ 10 unskinned and 5 skinned uncured and 10 skinned cured roots were stored at each temperature. The skinned uncured roots were each skinned in 2 places, and the skinned cured roots were each skinned in 3 places. In a few instances roots were removed from the experiments following the initial storage because of decay by *Rhizopus*.

The results obtained from cured and uncured skinned roots (table 9) corresponded closely. The percentage of infection at 29° C. and 74 percent relative humidity was less in cured roots (6 percent) than at 28° and 78 percent relative humidity in uncured roots (40 percent). In the third experiment (not in table 9), involving relative humidities of 63, 71, 82, 90, and 96 percent at 29°, infection occurred only at a relative humidity of 63 percent. It will be seen, therefore, that there is some variation in infection at the various humidities at 28° and 29°. The results, however, are consistent in showing that the lower relative humidities involve a greater hazard for infection.

The results of the four experiments at a temperature of 12° C. show heavy infection, ranging from 83 to 100 percent of the wounded areas at all four humidities. There was no consistent relation of infection to the relative humidity at 12°.

RELATION OF TEMPERATURE AND HUMIDITY TO NORMAL INFECTION IN UNCURED SWEETPOTATOES

One experiment was conducted in which uncured unskinned sweetpotatoes were stored at harvest time at 15 different combinations of temperature and relative humidity (table 9) for 10 days and then stored at a common storage temperature of 15.5° C. and a relative humidity of 87 percent for 154 days. The roots were carefully selected for their freedom from wounds and other defects. Most of the infections occurred through broken secondary rootlets, some through slight abrasions of the skin, and some through sprouts. The results correspond closely with those obtained in roots artificially skinned before being exposed to the initial temperatures and humidities. No infection occurred in roots initially stored at temperatures ranging from 25° to 36.5° C. There was a tendency for the percentage of roots infected, as well as the average number of lesions per root, to increase with the lowering of the temperature (combined with high humidity) from 25° to 12°, although infection was heavier at 21° than at 19°. Again, there was a tendency for the percentage of infection, as well as the average number of lesions per root, to increase with the lowering of the relative humidity at 28°, although there was less infection at 59 than at 69 percent relative humidity. One hundred percent of the roots stored at each of the four relative humidities at 12° became infected. There was little relation between the average number of lesions per root and the relative humidity at which they were initially stored, although the average number of lesions per root was larger at the 2 lower humidities than at the 2 higher.

All the results of surface rot experiments show: (1) That the initial 10 days' storage largely governed infection with surface rot; (2) that the temperatures and relative humidities favorable for suberization and periderm formation limited infection; and (3) that the conditions of temperature and relative humidity that retarded or prevented suberization and periderm formation permitted of various amounts of infection with surface rot, depending upon the inhibition of the rate of wound-cork formation.

FUSARIUM END ROT

The rare infection by *Rhizopus* of roots that had been wounded either by cutting or by breaking off one tip end provided an unusual opportunity to study the effect of temporary storage at various

combinations of temperature and relative humidity followed by subsequent storage at a common temperature and relative humidity on healing and on invasion of sweetpotato tissue by fungi such as forms of *Fusarium*, *Penicillium*, *Alternaria*, etc. Infection by *Rhizopus* also was sufficiently limited as a result of temporary storage of roots bruised at one tip end to permit a study of infection as related to temperature and humidity of the initial storage.

Five experiments dealing with different types of wounding and infection by *Rhizopus* were run during a period of two storage seasons (1928-29 and 1929-30). All three types of wounded roots were employed in each of four of the experiments, and roots bruised at tip and roots cut in cross section at tip were used in the fifth experiment. In one of the experiments uncured roots were used; in the other four, cured roots.

The results of three experiments, all run during different periods in the same season and involving roots from the same crop, are shown in tables 10 and 11.

TABLE 10.—Effect of temperature and humidity during 10 days' storage on invasion and infection by species of *Fusarium*, *Penicillium*, etc., of uncured and cured sweetpotatoes wounded at one tip end of the roots¹

Initial storage conditions			Bruised tip			Broken tip and cross section tip		
Temperature (° C)	Relative humidity	Roots used	Roots infected	Total depth of decay ²		Roots used	Roots infected	Total depth of decay
	Percent	Number	Percent	Mm		Number	Percent	Mm
36.5	95	9	0	0		13	8	85
31	95	9	0	0		20	5	25
28	96	10	0	0		17	0	0
25	91	10	30	62		20	0	0
21	91	10	10	6		19	5	73
19	89	10	0	0		21	24	20
16	89	8	0	0		18	33	27
12	93	9	0	0		20	50	153
28	96	10	0	0		17	0	0
28	90	7	0	0		20	0	0
28	78	9	0	0		20	0	0
28	69	10	0	0		20	15	49
28	59	10	0	0		19	21	59
12	93	9	0	0		20	50	153
12	86	10	0	0		14	29	35
12	75	10	10	30		20	55	71
12	65	5	0	0		20	70	228

CURED ROOTS (EXPERIMENT 2)								
Temperature (° C)	Relative humidity	Roots used	Roots infected	Total depth of decay ²		Roots used	Roots infected	Total depth of decay
	Percent	Number	Percent	Mm		Number	Percent	Mm
37	93	4	0	0		8	13	40
31	94	4	0	0		10	10	10
29	96	3	0	0		10	20	37
25	94	5	20	12		10	0	0
22	93	4	0	0		15	13	0
21	90	4	0	0		5	0	0
15.5	92	3	0	0		10	60	167
12	93	2	0	0		10	90	36
29	96	3	0	0		10	20	37
29	90	5	0	0		8	13	23
29	82	5	0	0		9	0	0
29	71	5	0	0		10	20	10
29	63	5	0	0		10	40	182
12	93	2	0	0		10	90	36
12	85	4	100	45		10	100	157
12	76	5	80	140		10	100	358
12	63	5	0	0		10	100	447

¹ The uncured roots were harvested Oct. 11, 1929, wounded immediately, and stored at various initial temperatures and relative humidities for 10 days, after which they were stored at a temperature of 15.5° C. and a relative humidity of 87 percent for 154 days. The cured roots were harvested at the same time, cured for 10 days at a temperature of 29° and a relative humidity of 74 percent, exposed, after being wounded, to initial temperatures and humidities for 10 days, and then stored for 142 days at 12.5° and a relative humidity of 58 percent.

² Total depth of decay includes decay in all roots.

TABLE 11.—*Effect of temperature and humidity during 10 days' storage on invasion and infection by species of Fusarium, Penicillium, etc., of cured sweetpotatoes wounded at the tip end of the roots*¹

Initial storage conditions		Infection following indicated type of wounding (experiment 3)									
		Bruised tip					Cross section tip				
Temperature (° C.)	Relative humidity	Roots infected		Roots used	Total penetration of decay ²		Average penetration		Extremes of penetration		Total penetration of decay ²
		Number	Percent		Mm	Mm	Number	Percent	Number	Percent	
31	94	5	20	6	1 2	6	5	0	0	0	Mm 0
29	96	3	0	0	0	0	5	1	0	0	0
25	94	3	66	11	3 7	5-6	5	0	0	0	9
21	83	2	100	28	14.0	10-15	5	0	0	0	0
17	80	4	100	20	3.0	4-7	5	1	0	0	0
15.5	95	4	100	21	5.3	3-6	4	4	100	15	6
12	64	5	0	0	0	0	5	5	100	39	2-5
29	96	4	25	12	3 3	12	5	1	20	9	4-19
28	80	3	33	7	2 3	1	5	0	0	0	0
28	78	3	0	0	0	0	5	1	20	3	0
28	69	5	0	0	0	0	5	0	0	0	0
28	62	5	0	0	0	0	5	0	0	0	0
13	85	4	100	41	10 3	2-20	5	5	100	39	4-19
12	85	4	100	49	24 8	4-80	6	6	100	218	4-81
12	76	4	100	27	6 8	3-11	5	5	100	228	7-97
12	64	4	100	27	6 8	3-11	2	2	100	19	8-11

¹ These roots were harvested Oct. 11, 1929, cured 10 days at a temperature of 29° C. and a relative humidity of 74 percent, and stored at a temperature of 10° to 15° and a relative humidity of 65 to 80 percent until Jan. 23, 1930, the date of the initiation of this experiment. The roots were then wounded and stored for an initial period of 10 days at various temperatures and relative humidities and for a subsequent period of 30 days at 13° and 50 percent relative humidity and of 147 days at 15.5° and 87 percent relative humidity.

² Total penetration of decay includes decay in all roots.

The data obtained from the two types of wounding, broken tip and cross section tip, were so similar that the results from experiments 1 and 2 were summarized together in table 10. The results from these two types of wounding were consistent in showing either no infection or comparatively small percentages of infection following exposure to initial temperatures above 19° C. combined with high humidity, the highest individual percentage being 20 at 29° and 96 percent relative humidity (cured roots, table 10). As the initial storage temperature fell below 21° there was in most instances a tendency for the percentage of infection to increase (tables 10 and 11). Infection took place readily in roots subjected to all four humidities at a temperature of 12°. The distribution of infection as related to the various initial relative humidities at 28° and 29° was erratic.

The results obtained when the roots were bruised at one of the root tips are not quite so consistent in their relation to the conditions of initial storage, although, in the main, infection took place more readily in roots subjected to initial storage conditions unfavorable for healing. There is a tendency for bruised tissue to dry out in roots that escape infection by *Rhizopus*. It is possible that the drying out may impede the activities of such fungi as *Fusarium*, *Penicillium*, etc., during the period of subsequent storage long enough to permit the process of healing to go on to completion in many roots if the environmental conditions of subsequent storage are favorable.

Out of 193 isolations made from roots infected through these wounded tip ends, 46 percent yielded forms of *Fusarium*, mostly of the *elegans* group; 27 percent, *Penicillium*; 4 percent, *Alternaria*; 1 percent, unidentified fungi; 0.5 percent, *Rhizopus tritici*; 0.5 percent, *R. nigricans*; 1 percent, *Mucor*; and 21 percent were sterile. Most of the lesions resembled fusarium end rot. It is believed that *Penicillium* invasion was largely secondary because it was never the soft watery type characteristic of the rot normally produced in sweet-potatoes by forms of *Penicillium*. It is believed that the foregoing data throw some light on the occurrence of fusarium end rot in storage. Its occurrence has remained more or less obscure largely because most of the efforts to produce it by artificial inoculation have failed. A partial explanation of this failure is probably that following inoculation the roots were stored under conditions that favor healing. The condition that apparently favors infection is a prolonged inhibition of healing. Associated with an inhibition of healing is an apparent deterioration of host tissue in the wounded areas. It is possible that the deteriorated tissue may assist the forms of *Fusarium* in establishing themselves in the host tissue.

JAVA BLACK ROT

RELATION OF TEMPERATURE TO GROWTH OF *DIPLODIA TUBERICOLA*

To determine the influence of temperature on the growth of *Diplodia tubericola* an experiment was conducted in which 200-ml Erlenmeyer flasks, each containing 40 ml of carrot agar, were inoculated by placing at the center of the agar a small bit of fungus growth, including pycnidia and spores, from a corn-meal culture of *D. tubericola*. Ten flasks were placed at each of a number of temperatures (table 12), from 2° to 42° C. All measurements of the diameter of

the colonies taken before the growth reached the borders of the agar were taken with calipers that were extended down into the flasks.

No growth occurred in 16 days at temperatures of 40° or 42° C., nor in 35 days at 8°. The maximum amount of growth in 1 day occurred at 31°. Only slightly less growth occurred at 29°. There was a gap of 6° between temperatures 31° and 37°. It is possible that the rate of growth may have been greater at temperatures within this gap had such temperatures been employed. The fact that the amount of growth at 29° was nearly as great as at 31° and that there was a marked drop in the amount of decay as the temperature rose to 37° would seem to indicate that 31° was near the optimum temperature for the growth of *Diplodia tubericola* on culture media. The retardation of growth was pronounced as the temperature rose above 31° or fell below 29°.

TABLE 12 · Influence of temperature on the growth of *Diplodia tubericola* on carrot agar

Temperature (° C.)	Average area of colonies after—			
	1 day	3 days	7 days	11 days
	<i>Mm</i> ²	<i>Mm</i> ²	<i>Mm</i> ²	<i>Mm</i> ²
42.....	0	0	0	10
40.....	0	0	0	10
37.....	800			
31.....	2,049			
29.....	2,004			
25.....	866			
21.5.....	547			
19.....	202			
15.....	(?)	203		
13.....	0		210	
8.....	0	0	0	30

¹ There was no growth at 40° and 42° in 16 days.

² Growth just started.

³ There was no growth at 8° or below in 35 days.

RELATION OF WOUNDING TO INFECTION

No experiments were conducted that were especially designed to determine the effect of wounding on infection by *Diplodia tubericola* or to test the capacity of the pathogene to penetrate the unbroken skin. In the experiments discussed below, the uninjured skin of many roots was exposed to inoculum of the pathogene under conditions that readily induce infection in wounded tissue. The results obtained show that normally the fungus does not cause infection through the uninjured skin. *D. tubericola* sometimes invades lesions produced by species of *Rhizopus* and by *Mucor racemosus*, and possibly other lesions.

TECHNIC EMPLOYED IN STUDY OF RELATION OF TEMPERATURE AND HUMIDITY TO INFECTION BY DIPLODIA TUBERICOLA IN WOUNDED SWEETPOTATOES

The technic employed in the infection experiments with *Diplodia tubericola* and *Sclerotium bataticola* was developed as a result of the experience gained in the experiments already discussed.

Unlike *Rhizopus tritici*, *R. nigricans*, and *Fusarium oxysporum*, *Diplodia tubericola* commonly is not found associated with sweetpotatoes grown in the vicinity of Washington, D. C. Only an occasional infection has been observed to occur normally. In order to

insure uniform infection, wounded roots (cross section tip)¹¹ were dipped in a macerated culture of the fungus grown on corn meal. This method of inoculation insured the presence of the pathogene on the wounded area and at the same time avoided rewounding during the process. Such a precaution was necessary in the wound-healing studies that follow. This method of wounding lent itself admirably to the study of the environmental conditions under which infection occurred. Infection by the species of *Rhizopus* in this type of wounding was rare, and the slowness with which invasion by *Fusarium* occurred enabled such pathogenes as *Diplodia tubericola* and *Sclerotium bataticola*, when present, to establish themselves in such unhealed wounds whenever the temperature and humidity were favorable.

INFECTION IN SWEETPOTATOES INOCULATED WITH *DIPLODIA TUBERICOLA* WHEN FRESHLY WOUNDED AND IN THOSE INOCULATED AFTER 10 DAYS' STORAGE AT VARIOUS TEMPERATURES AND HUMIDITIES FOLLOWING WOUNDING

Five experiments were conducted which were designed to yield information regarding the following questions: (1) Do sweetpotatoes change during the storage season in their susceptibility to infection by *Diplodia tubericola*? (2) What conditions of temperature and humidity favor infection of freshly wounded roots? (3) What effect does 10 days' storage of wounded roots at various temperatures and relative humidities before inoculation have on infection during subsequent storage following inoculation?

The Yellow Jersey variety was used in all five experiments. The treatment the roots received before and during the experiments was, briefly, as follows:

In experiment 1 the roots were harvested October 6, 1928, cured for 10 days at a temperature of 22° to 26° C. and a relative humidity of 50 to 82 percent, and then stored at a temperature of 10° to 15° and a relative humidity of 70 to 85 percent until February 13, 1929, when the experiment was started. The roots were inoculated when freshly wounded (cross section tip) and 10 roots each were stored at 15 combinations of temperature and relative humidity for 46 days.

Experiment 2 was started February 25, 1929, with roots from the stock used in experiment 1. (1) A quantity of roots were inoculated when freshly wounded (cross section tip) and stored 10 each at the 15 combinations of temperature and relative humidity given in table 13 and in addition at a temperature of 15.5° C. and a relative humidity of 87 percent and at 21° and 89 percent relative humidity for 45 days. (2) A quantity of roots were wounded, then stored 10 each at 15 combinations of temperature and relative humidity for 10 days, and then inoculated; after inoculation these roots were further treated as follows: (a) Roots from each initial temperature and relative humidity were returned to the conditions of temperature and relative humidity from which they came for 35 days' further storage, and (b) roots from each initial storage condition were stored at 15.5° and 87 percent relative humidity and at 21° and 89 percent relative humidity for 35 days.

In experiment 3 roots from the stock used in experiments 1 and 2 were wounded March 5, 1929, by cutting a thin slice (longitudinal section shallow p. 295) from the side of each root. After wounding, the procedure was the same as in experiment 2, except that the subse-

¹¹ The roots were wounded by cutting off one tip end of each root at a point where the root was about 1 cm in diameter.

quent storage at 15.5° C. and 87 percent relative humidity was omitted and the storage at the other conditions lasted only 26 days.

In experiment 4 roots harvested October 11, 1929, were wounded and inoculated immediately without curing, and stored 10 each at the various temperatures and relative humidities shown in table 13, for 61 days.

In experiment 5 uncured roots from the stock used in experiment 4 were subjected to the same treatment and procedure, except the duration of subsequent storage (table 13), as those in experiment 2, five roots being used at each storage condition.

TABLE 13.—*Infection of sweetpotatoes by Diplodia tubericola during various periods of storage following different treatments*¹

Initial storage conditions		Infection in roots inoculated							
		When freshly wounded (after inoculation roots stored for 21 days at initial storage conditions only)		After 10 days at initial storage conditions following wounding					
				Subsequent storage for 51 days at conditions same as initial		Subsequent storage for 51 days at 15.5° C. and 87 percent relative humidity		Subsequent storage for 51 days at 21° C. and 89 percent relative humidity	
Temperature (° C.)	Relative humidity	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay
	Percent	Percent	Mm	Percent	Mm	Percent	Mm	Percent	Mm
36.5	95					0	0	0	0
31	95	80	218	0	0	0	0	0	0
28	96	80	87	0	0	0	0	0	0
25	91	80	25	0	0	20	2	0	0
21	91	80	17	0	0	20	40	0	0
19	89	100	25	20	8	40	16	0	0
16	89	60	14	60	13	60	17	40	115
12	93	40	6	0	0	60	12	40	170
28	96	80	87	0	0	0	0	0	0
28	90	100	50	0	0	0	0	20	3
28	78	60	13	0	0	20	17	20	15
28	69	60	105	0	0	0	0	40	10
28	59	40	127	20	25	² 50	33	40	42
12	93	40	6	0	0	60	12	40	170
12	86	60	8	40	9	80	18	20	45
12	75	100	23	80	40	100	54	100	63
12	65	60	61	100	74	³ 66	46	100	91
(check (roots inoculated when freshly wounded))						100	103	80	114

¹ 5 roots were used for each condition of storage except in the cases noted. All roots were wounded by cutting off the tip end.

² 6 roots were used instead of 5.

³ 3 roots were used instead of 5.

Whenever the treatment and storage conditions were comparable the results of all five experiments were so similar that only those of experiment 5 are presented in table 13.

The results of the five experiments, together with some ¹² discussed later (p. 311), show that if cured and uncured roots are wounded in the same manner and the subsequent treatment as to inoculation and storage is the same there is no apparent difference in their susceptibility to infection by *Diplodia tubericola*. Infection occurred under

¹² The results from these later experiments were obtained from cured roots from the same stock as the uncured roots in experiment 5. One of these experiments was started Nov. 26, 1929, following curing, and one Jan. 13, 1930.

the same conditions and in similar proportions in cured and uncured roots.

A certain percentage of infection by *Diplodia tubericola* occurred at all conditions of temperature and humidity in roots inoculated when freshly wounded (table 13). The tendency for percentage of infection to drop at temperatures below 19° C. was also evident in the other experiments (not shown in table 13). There was a tendency for the total depth of decay to increase with the rise in temperature from 12° to 31°.

There seems to be little relation of humidity to the percentage of infection at 28° C. in roots inoculated when freshly wounded, although the results in table 13 suggest a possible relation. In 2 of the 5 experiments there was no infection at a relative humidity of 96 percent, and about an equal percentage of infection occurred at the other humidities. At a temperature of 12° there was a tendency for the percentage of infection as well as the total depth of decay to increase with the lowering of the relative humidity, particularly in the early stages of infection. In this experiment and in one other (table 13) the percentage of infection increased with the lowering of the relative humidity from 93 to 73 percent, then dropped off as the humidity fell to 65 percent. A temperature of 12° has not been found very favorable to the continuation of decay by *Diplodia tubericola*. In a large percentage of the cases where infection began with this pathogene, the organism was displaced by species of *Fusarium* and *Penicillium*.

In roots wounded and stored for 10 days at the various temperatures and humidities, then inoculated and returned to the temperatures and humidities from which they came for 51 days' further storage, infection had a very definite relation to the conditions of initial storage that are either unfavorable to suberization and periderm formation or retard their formation. No infection occurred at temperatures of 21°, 25°, 28°, and 31° C. combined with high humidity. Passing from a temperature of 21° to 19° and 16°, the percentage of infection as well as the total penetration increased. At a temperature of 12° there was no infection at a relative humidity of 93 percent. In 3 out of the 4 other experiments (data not included in table 13) there was infection at 12° (combined with the highest humidity employed); in 2 of these 3 there was no infection at temperatures above 12°. Infection took place readily at the three lower humidities at 12°. At 28° only relative humidities of 59 percent in this experiment (table 13) and 63 percent in another experiment, of the 5 employed, were sufficiently unfavorable to healing to permit of infection. Infection was obtained at 59 percent relative humidity in two other experiments.

If subsequent storage, following 10 days' initial storage at various temperatures and humidities, influences infection and decay, it does so in the case of roots stored at initial combinations of temperature and relative humidity that either prevented or retarded suberization or periderm formation. Subsequent storage conditions favorable to cork formation usually resulted in less infection than conditions unfavorable to cork formation in roots initially stored under conditions of temperature and humidity unfavorable to healing. The results have

been consistent in showing that whenever the conditions of temperature, humidity, and time were such as to permit cork formation to go on to completion before the roots were inoculated infection did not result under the conditions of subsequent storage employed in these experiments. Storage for 10 days at temperatures of 28°, 31°, and 36.5° C. combined with high humidity at all the conditions of subsequent storage prevented infection. There was one infection at 25°, but decay made scarcely any progress. A subsequent storage at 15.5° and 87 percent relative humidity following initial storage at temperatures of 19°, 16°, and 12° (combined with high humidity) showed a higher percentage of infection, with one exception (table 13), than did roots that were returned to these initial temperatures or were subjected to subsequent storage at 21° and 89 percent relative humidity. In experiment 2 (not shown in table 13) a higher percentage of infection resulted during subsequent storage at 21° and 89 percent relative humidity. There was less infection (table 13) in roots initially stored at the various humidities at a temperature of 28° followed by subsequent storage under these same conditions than in roots with the same initial storage followed by subsequent storage at 15.5° and 87 percent relative humidity and at 21° and 89 percent relative humidity. In experiment 2 (not shown in table 13) there was no infection during subsequent storage at various relative humidities at 28° following an initial storage under the same conditions, except at 63 percent relative humidity, the lowest employed. With certain exceptions, there was a tendency for infection and decay to increase with the lowering of the relative humidities both at 28° and 12° in roots subsequently stored at 15.5° and 21°. In roots from the various initial temperatures and humidities the total decay was greater in the main in those subsequently stored at 21° than at 15.5°.

RELATION OF EXTREMES OF TEMPERATURE AND HUMIDITY DURING INITIAL STORAGE OF WOUNDED CURED SWEETPOTATOES TO INFECTION BY *DIPLODIA TUBERICOLA* DURING SUBSEQUENT STORAGE

Two experiments were conducted to compare the effects of 10 days' initial storage of wounded sweetpotatoes at high temperatures and relative humidities with those at low temperatures and humidities, during a subsequent storage at various temperatures and humidities. In experiment 1 Yellow Jersey roots were wounded (cross section tip) and stored for 10 days at 2 combinations of temperature and relative humidity (some at 29° C. and 97 percent and some at 13° and 69 percent), then inoculated with *Diplodia tubericola* and stored at 15 combinations of temperature and relative humidity (table 14), together with some roots inoculated when freshly wounded. Experiment 2 included the Puerto Rico variety in addition to Yellow Jersey. The initial storage temperatures and relative humidities were 26° and 92 percent and 13° and 59 percent in the case of Yellow Jersey, and 29° and 97 percent and 13° and 59 percent in the case of Puerto Rico. The subsequent storage temperature of 37° was omitted in experiment 2. Aside from the exceptions mentioned the two experiments were similar. The average results obtained from the Yellow Jersey variety in the two experiments are presented in table 14.

TABLE 14.—*Relation of temperature and humidity to infection and decay of sweet-potatoes (Yellow Jersey) by Diplodia tubericola during 10 to 12 days' storage following different treatments*¹

Final storage conditions		Roots inoculated when freshly wounded		Roots wounded and initially stored for 10 days at—			
				13° C. and relative humidities of 59 and 69 percent and then inoculated		26° and 29° C. and relative humidities of 92 and 97 percent and then inoculated	
Temperature (° C.)	Relative humidity	Roots infected	Average depth of decay	Roots infected	Average depth of decay	Roots infected	Average depth of decay
	Percent	Percent	Mm	Percent	Mm	Percent	Mm
37.....	93	20	0.6	20	0.6	0	0
30-31.....	94	90	17.0	90	25.0	0	0
29.....	97	100	26.0	70	29.0	0	0
25.....	92	80	15.0	90	23.0	0	0
22.....	91-95	60	18.0	60	5.0	0	0
18-19.....	88-94	30	.9	60	2.0	0	0
15.5.....	91-92	0	0	40	4.0	0	0
12.....	93-94	0	0	10	.3	0	0
29.....	97	100	26.0	70	29.0	0	0
29.....	89-90	90	41.0	80	32.0	10	9
29.....	80	100	38.0	100	27.0	0	0
29.....	69-72	80	17.0	80	20.0	10	8
29.....	60-63	50	24.0	60	24.0	0	0
12.....	93-94	0	0	10	.3	0	0
12.....	85	0	0	0	0	0	0
12.....	76-78	0	0	0	0	0	0
12.....	63-64	0	0	0	0	0	0

¹ The results recorded in this table except at 37° C. are the averages of two experiments. Five roots were used for each treatment and condition of temperature and relative humidity in each experiment. Results were taken after 10 and 12 days of final storage, respectively.

The initial storage of wounded roots for 10 days at high temperatures and high relative humidities (26° and 29° C. and 92 and 97 percent relative humidity, respectively) before inoculation excluded infection except at two conditions of subsequent storage (29° and a relative humidity of 89 to 90 percent, and 29° and a relative humidity of 69 to 72 percent) and here only 1 root out of 10 was involved in each instance (table 14). In roots initially stored at a low temperature and low humidity (13° and 59 and 69 percent) infection occurred at all subsequent storage conditions of temperature and relative humidity, except the three lowest humidities at 12°, within 10 to 12 days. In roots inoculated when freshly wounded infection occurred at temperatures of 18° and above, but not at 12° and 15.5°, in 10 to 12 days' subsequent storage. From the occurrence of infection and the amount of decay, it will be seen that there is no significant difference in susceptibility between roots inoculated when freshly wounded and roots inoculated after 10 days' storage at low temperature and relative humidity following wounding.

Results similar to the foregoing were obtained with roots of the Puerto Rico variety, wounded and initially stored for 10 days, in one instance at a temperature of 29° C. and relative humidity of 97 percent and in another at 13° and 59 percent relative humidity, followed by inoculation with *Diplodia tubericola* and 12 days' subsequent storage at 14 combinations of temperature and relative humidity.

RELATION OF TEMPERATURE TO RATE OF DECAY

The results obtained during 10 to 12 days' storage at various temperatures following inoculation from (1) roots inoculated when freshly wounded and (2) roots inoculated after 10 days' storage at a

temperature of 13° C. and relative humidities of 59 and 69 percent illustrate the effect of temperature on rate of decay (table 14 and fig. 1). There is not much difference in the rate of decay in roots with the two types of treatment, although the rate is as great or greater at all temperatures at which infection occurred, except at 22°, in roots subjected to 10 days' initial storage at 13° and 59 and 69 percent relative humidity (fig. 1, *B*) than in roots inoculated when freshly wounded (fig. 1, *A*). There was some variation in individual experiments in the amount of decay that took place at a given temperature, but the average of the two experiments following a given type of treatment produces fairly uniform curves. If averages of the two types of treatment are taken, a still smoother curve is obtained (fig. 1, *C*). The greatest amount of decay occurred at 29° in the experiments reported in table 14, but in another experiment it occurred at 31° (table 13). As the temperature was raised above or lowered below 29° the rate of decay rapidly declined.

The data in table 15, obtained from one of the experiments discussed in connection with table 14, illustrate roughly the rate at which infection and decay by *Diplodia tubericola* occur at temperatures of 12°, 15.5°, and 17.7° C. following preliminary treatment and inoculation, as indicated. It will be noted that decay takes place very

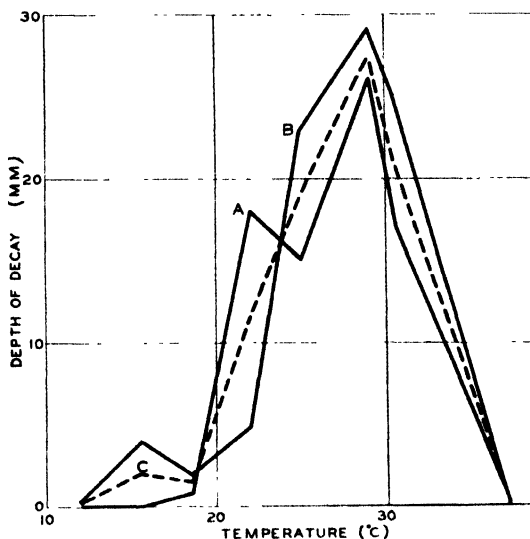


FIGURE 1—Depth of decay in sweetpotatoes caused by *Diplodia tubericola* at various temperatures. *A*, Roots inoculated when freshly wounded; *B*, wounded roots stored for 10 days at 13° C. and at 59 and 69 percent relative humidity before inoculation; *C*, average of *A* and *B*.

slowly, the average penetration being slight even after 44 days' storage at these temperatures. The slowness in the advance of decay is due in part, it is believed, to the retarded activity of the pathogene at these temperatures. *Diplodia tubericola* is often displaced by other organisms, especially *Fusarium*, at these temperatures, particularly at 12°. The high average depth of decay (13 mm) after 44 days' storage at 12° and 63 percent relative humidity in roots inoculated after wounding and 10 days' storage at 13° and 59 percent relative humidity was due to deep decay (55 mm) in one root rather than to a consistent advance in all the roots. Incidentally it will be noted that the roots with an initial storage temperature of 26° and a relative humidity of 92 percent showed scarcely any infection after 44 days' storage at the temperatures 12°, 15.5°, and 17.7° and the various relative humidities at 12°, or after 16 days' further storage at 21° and 89 percent relative humidity. Nor did these roots show signs of infection by other organisms.

TEMPERATURE LIMITS OF INFECTION

No experiments designed to determine the exact temperature limits of infection for *Diplodia tubericola* were conducted.

The slowness of infection and decay at 12° C., and the fact that *Diplodia tubericola* is largely displaced by other organisms and that there was no growth on carrot agar at 8° after 35 days' storage (table 12) indicate that 12° is near the lower temperature limit of infection and decay for this fungus.

The slowness in rate of decay by this fungus at 37° C. and the fact that there was no growth of the pathogene in 16 days at 40° (table 12) show that 37° is near the maximum temperature for infection and decay.

EFFECT OF DURATION OF INITIAL STORAGE OF WOUNDED SWEETPOTATOES ON INFECTION BY DIPLODIA TUBERICOLA DURING SUBSEQUENT STORAGE

An experiment was conducted in which roots of the Yellow Jersey and Nancy Hall varieties were wounded as in the preceding cases, stored at a temperature of 25.5° C. and a relative humidity of 94 percent for various periods, then inoculated with *Diplodia tubericola* and returned to the same temperature and relative humidity for 13 and 22 days' further storage. The initial storage periods for the Yellow Jersey were 2, 4, 6, 8, and 10 days, respectively, and for Nancy Hall 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days, respectively. In order to use the same inoculum for all the roots, those for the 10-day initial storage period were wounded first, those for the 9-day period next, etc., each lot being wounded and stored on successive days in the order of time assigned to the initial storage. At the end of the various initial storage periods all the roots, including the freshly wounded ones, were inoculated at the same time with the same inoculum.

No infection occurred in either Yellow Jersey or Nancy Hall roots subjected to an initial storage of more than 4 days (table 16).

TABLE 16.—*Effect of duration of initial storage of wounded sweetpotatoes followed by inoculation with Diplodia tubericola on infection and decay during 2 periods of subsequent storage*¹

Duration of initial storage (days)	Infection and decay in the Nancy Hall variety after storage periods indicated								Infection and decay in Yellow Jersey variety after storage period indicated			
	Initial, 29° C. and 96-percent relative humidity; subsequent, 28° and 89 percent relative humidity				Initial and subsequent, 25.5° C. and 94 percent relative humidity				Initial and subsequent, 25.5° C. and 94 percent relative humidity			
	13 days		28 days		13 days		22 days		13 days		22 days	
	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay
	Percent	Mm	Percent	Mm	Percent	Mm	Percent	Mm	Percent	Mm	Percent	Mm
0.....	100	153	100	149	100	55	100	58	80	15	100	17
1.....	80	29	100	3	80	14	100	24
2.....	0	0	20	2	40	5	60	13	20	4	60	7
3.....	0	0	0	0	20	2	60	10
4 ²	0	0	0	0	0	0	0	0	0	0	20	2

¹ 5 roots were used for each time interval.

² The apparent decrease in depth of decay in 28 days as compared to 13 days is due to error of measurement. The measurements taken after 13 days were from the outside of the root and those taken after 28 days were from the interior after the roots had been halved. However, there was no advance of decay after 13 days.

³ There was no infection after initial storage periods of 5 days or more.

In another experiment, roots of the Nancy Hall variety were stored for periods of 1 to 10 days at a temperature of 29° C. and a relative humidity of 96 percent and then inoculated and stored at 28° and 89 percent relative humidity for 13 and 28 days (table 16). The wounding and inoculation were made in the same manner as in the preceding experiment. No infection occurred in roots that had had an initial storage period longer than 2 days.

CHARCOAL ROT

RELATION OF TEMPERATURE TO GROWTH OF *SCLEROTIUM BATATICOLA* ON CARROT AGAR

In order to determine the temperature limits and optimum temperature for growth of *Sclerotium bataticola*, as well as the rate of growth at various temperatures, ten 200-ml Erlenmeyer flasks, each containing 40 ml of carrot agar, were inoculated with *S. bataticola* and stored at each of 11 temperatures (table 17). The maximum amount of growth in 1 day occurred at a temperature of 31° C. As the temperature rose above 31° or fell below it the rate of growth rapidly declined. *S. bataticola* showed slight growth in 3 out of 10 flasks at 42° in 16 days and slight growth in 2 out of 10 flasks at 8° in 35 days in contrast to an absence of growth by *Diplodia tubericola* at these temperatures in the same periods.

TABLE 17.—Influence of temperature on the growth of *Sclerotium bataticola* on carrot agar

Temperature (°C.)	Average area of colonies after—				Temperature (°C.)	Average area of colonies after—			
	1 day	3 days	7 days	11 days		1 day	3 days	7 days	11 days
	Mm ²	Mm ²	Mm ²	Mm ²		Mm ²	Mm ²	Mm ²	Mm ²
42 0	0	0	0	1 0	21 5	5 1			
40 0	0	0	0	1 0	19 0	(2)	1, 005		
37 0	511				15 0	0	(2)	3, 341	
31 0	2, 100				13 0	0	0	210	
29 0	580				8 0	0	0	0	3 0
25 0	207								

¹ Growth had just started in 3 out of 10 flasks at 42° C. and in 1 out of 10 flasks at 40° C. in 16 days.

² Growth just started

³ There was slight growth in 2 flasks out of 10 at 8° C. in 35 days.

RELATION OF WOUNDING TO INFECTION

Sclerotium bataticola seems quite dependent upon wounds for infection. It sometimes invades lesions of mottled necrosis, *Rhizopus* soft rot, lesions produced by *Mucor racemosus*, and possibly other lesions. Injury by heat, such as that produced in the field when the roots lie in the sun, seems to open the way for infection in fields heavily infested with *S. bataticola*. However, mechanical wounding remains the major preliminary condition essential to infection. If the unbroken skin is exposed to inoculum of *S. bataticola*, infection rarely if ever results. This is shown by the infection experiments discussed below, conducted under the same conditions and involving the same technic as those carried on in connection with *Diplodia tubericola*.

INFECTION BY *SCLEROTIUM BATATICA* IN SWEETPOTATOES INOCULATED WHEN FRESHLY WOUNDED AND IN THOSE INOCULATED AFTER 10 DAYS' STORAGE AT VARIOUS TEMPERATURES AND HUMIDITIES FOLLOWING WOUNDING

The materials (except the pathogene) and the technic employed in these experiments (five in number) were the same as in the experiments with *Diplodia tubericola*. The experiments were run concomitantly and under the same conditions of temperature and relative humidity.

The results of the five experiments were so similar whenever the conditions of treatment and storage were parallel that the results of only one are presented (table 18). In this experiment uncured roots obtained directly from the field at harvest time (Oct. 10, 1929) were used. One lot was held for 10 days at 15.5° C. and 87 percent relative humidity (noncuring condition), then wounded and inoculated with *Sclerotium bataticola*. Five of these roots (roots inoculated when freshly wounded) were then stored 21 days at each of the 15 combinations of initial temperature and relative humidity given in table 18. Another lot of roots were wounded immediately after harvesting, and part of them were stored for 10 days at each of the 15 initial temperatures and relative humidities given in table 18. At the end of this 10 days' storage all the roots were inoculated. Five of these roots from each initial storage condition were stored as follows: (1) For 21 days at each of the initial storage conditions; (2) for 51 days at 15.5° and 87 percent relative humidity; and (3) for 51 days at 21° and 89 percent relative humidity. Roots inoculated when freshly wounded were also stored under the two last-mentioned conditions of storage as checks. The same inoculum was used throughout, and all the roots were inoculated at one time.

TABLE 18.—*Infection of sweetpotatoes by Sclerotium bataticola during various periods of storage following different treatments*¹

Initial storage conditions		When freshly wounded (after inoculation roots stored for 21 days at initial storage conditions)		Infection in roots inoculated							
				After 10 days' storage at initial temperatures and relative humidities following wounding							
				Subsequent storage (for 51 days) at conditions same as initial		Subsequent storage (for 51 days) at 15.5° C. and 87 percent relative humidity		Subsequent storage (for 51 days) at 21° C. and 89 percent relative humidity			
Temperature (° C.)	Relative humidity	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay
		Percent	Mm	Percent	Mm	Percent	Mm	Percent	Mm	Percent	Mm
36.5	95	80	307	0	0	0	0	20	150	0	0
31.0	95	80	337	20	100	0	0	0	0	0	0
28.0	96	80	202	0	0	0	0	0	0	0	0
25.0	91	100	262	0	0	0	0	0	0	0	0
21.0	89	100	32	0	0	20	15	0	0	0	0
19.0	89	100	10	0	0	20	8	0	0	0	0
16.0	89	60	7	0	0	60	26	80	131	0	0
12.0	93	40	7	0	0	100	25	100	310	0	0
28.0	96	60	337	0	0	0	0	0	0	0	0
28.0	90	80	134	0	0	0	0	0	0	0	0
28.0	78	100	346	0	0	40	25	40	48	0	0
28.0	69	100	195	40	117	20	3	20	110	0	0
28.0	59	80	248	20	115	20	2	80	16	0	0
12.0	93	40	7	0	0	100	25	100	310	0	0
12.0	86	40	10	20	10	100	46	100	132	0	0
12.0	75	0	0	100	43	80	42	100	281	0	0
12.0	65	20	3	100	62	80	27	80	138	0	0
Check (roots inoculated when freshly wounded)						100	46	100	390		

¹ 5 roots were used for each condition of storage except in the case of roots inoculated when freshly wounded and stored at 15.5° C. and 87 percent relative humidity and at 21° C. and 89 percent relative humidity, in which cases 7 and 10 roots, respectively, were used. All roots were wounded by cutting off 1 tip end of each.

In roots inoculated when freshly wounded, infection by *Sclerotium bataticola* took place readily at temperatures from 19° to 31° C., and at all relative humidities at 28° (table 18). At temperatures below 19° (see also table 19) infection was more uncertain and at 12° *Sclerotium* was generally displaced by other organisms, especially species of *Fusarium*. There seems to be little relation between the percentage of infection and relative humidity at either 28° (see also table 19)¹³ or 12°.

An initial 10 days' storage of wounded roots at various temperatures and relative humidities altered materially the infection with *Sclerotium bataticola* that occurred when they were inoculated and stored at the same temperatures and relative humidities as the roots inoculated when freshly wounded. With the exception of 1 root at 31° C., no infection occurred in roots stored for 51 days at any of the temperatures combined with high humidity. There was no infection at temperatures of 12° and 16° combined with high humidity in the experiment reported in table 18, but there was in 2 other experiments at 16°, and in 1 of the 2 at 12° (not included in table 18). The handicaps that these conditions impose upon healing and the activities of the pathogene were such that a struggle for supremacy resulted. Some infection occurred at the 2 lowest relative humidities (59 and 69 percent) at 28° and at the 3 lowest humidities (65, 75, and 86 percent) at 12°. These results show that healing is not always sufficiently complete by the end of 10 days' storage at the lower humidities at 12° and 28° to preclude infection.

Judged by the infection with *Sclerotium bataticola* that occurred (table 18) during subsequent storage at 15.5° C. and 87 percent relative humidity and at 21° and 89 percent relative humidity, healing was not always complete by the end of 10 days' initial storage at temperatures of 12° to 21° (combined with high humidity), at the three lowest humidities at 28°, or at any of the humidities at 12°.¹⁴ The rate of decay was consistently higher at a subsequent temperature of 21° than at 15.5°. Infection occurred following subsequent storage at 15.5° after initial temperatures of 12° to 21°, whereas infection occurred following subsequent storage at 21° after initial temperatures of 12° to 16°. These results (at 15.5° and 21°) were almost reversed in another experiment, in which, however, the subsequent relative humidity at 15.5° was 95 percent instead of 87 percent. In wounded roots inoculated with *Sclerotium bataticola* after a day's initial storage at temperatures from 12° to 21°, infection during subsequent storage would seem to depend upon (1) whether wound healing is complete, (2) the stage of healing, and (3) the temperatures and relative humidity of subsequent storage. If wound healing had reached an advanced stage, it would seem possible that it might become complete before infection could occur, especially if the conditions of subsequent storage were favorable to healing; if unfavorable, infection might take place.

¹³ The results in table 18 indicate a possible relation between the percentage of infection and relative humidity at 28° C., but when all the results dealing with infection in freshly wounded roots are considered there seems to be no relation.

¹⁴ The one infection in roots with an initial storage at 33.5° C. and a subsequent storage at 21° may easily have been accidental. However, it may have been associated with the same factor that makes sweet potatoes more susceptible to decay by *Rhizopus tritici* (11) at temperatures above 33°.

RELATION OF EXTREMES OF TEMPERATURE AND HUMIDITY DURING INITIAL STORAGE OF WOUNDED CURED SWEETPOTATOES TO INFECTION BY *SCLEROTIUM BATATICOLA* DURING SUBSEQUENT STORAGE

The two initial storage temperatures and relative humidities in the two experiments reported in table 19 were selected to compare the effects of storing wounded roots at conditions very favorable to healing with the effects of storing at conditions that prevent healing.

TABLE 19.—*Influence of temperature and humidity on infection by Sclerotium bataticola in cured sweetpotatoes inoculated when freshly wounded and in sweetpotatoes wounded and stored for 10 days at each of two combinations of temperature and humidity and then inoculated*¹

Final storage conditions		Roots inoculated when freshly wounded		Roots wounded and initially stored for 10 days at—			
				13° C. and relative humidities of 59 and 69 percent and then inoculated		26° and 29° C. and relative humidities of 92 and 97 percent and then inoculated	
				Roots infected	Average depth of decay	Roots infected	Average depth of decay
Temperature (° C.)	Relative humidity	Roots infected	Average depth of decay	Roots infected	Average depth of decay	Roots infected	Average depth of decay
	Percent	Percent	Mm	Percent	Mm	Percent	Mm
37	93	80	11	80	13	0	0
30-31	94	90	77	100	50	0	0
29	97	100	87	100	40	20	9
25	92	100	54	90	21	0	0
22	91-95	88	22	100	28	0	0
18-19	88-94	40	9	50	3	0	0
15-5	91-92	30	1	60	6	0	0
12	93-94	0	0	0	0	0	0
29	97	100	87	100	40	20	9
29	89-90	90	64	80	24	10	9
29	80	90	77	90	57	0	0
29	69-72	100	55	90	16	10	7
29	60-63	100	61	70	19	0	0
12	93-94	0	0	0	0	0	0
12	85	0	0	0	0	0	0
12	76-78	0	0	0	0	0	0
12	63-64	0	0	0	0	0	0

¹ The results recorded are the averages of 2 experiments, 5 roots were used for each treatment and combination of temperature and relative humidity. The length of the final storage period in the experiments was 10 and 12 days, respectively.

The material used, aside from the pathogene, the technic employed in setting up the two experiments, and the initial and final storage conditions were the same as those employed in the same type of experiments in connection with Java black rot. The results reported are the average results of the two experiments.

Infection took place under the same conditions of temperature and relative humidity in final storage (table 19) both with roots inoculated when freshly wounded and with wounded roots inoculated after 10 days' storage at a temperature of 13° C. and relative humidities of 59 and 69 percent. The temperature of the storage following inoculation was the controlling factor governing the percentage of infection and the amount of decay. No infection occurred at any of the relative humidities at 12°, whereas at 29° it occurred at all the relative humidities.

Notwithstanding the heavy inoculation following 10 days' initial storage at temperatures of 26° and 29° and at relative humidities of 92 and 97 percent, very little or no infection occurred at any of the

subsequent storage conditions, indicating fairly complete protection due to formation of wound cork at the favorable temperature and relative humidity of the storage chamber.

RELATION OF TEMPERATURE TO RATE OF DECAY

The effect of temperature on the rate of decay is shown by the average depth of decay obtained in cured sweetpotatoes inoculated with *Sclerotium bataticola* when freshly wounded and from roots initially stored for 10 days at a temperature of 13° C. and relative humidities of 59 and 69 percent before inoculation (table 19). In

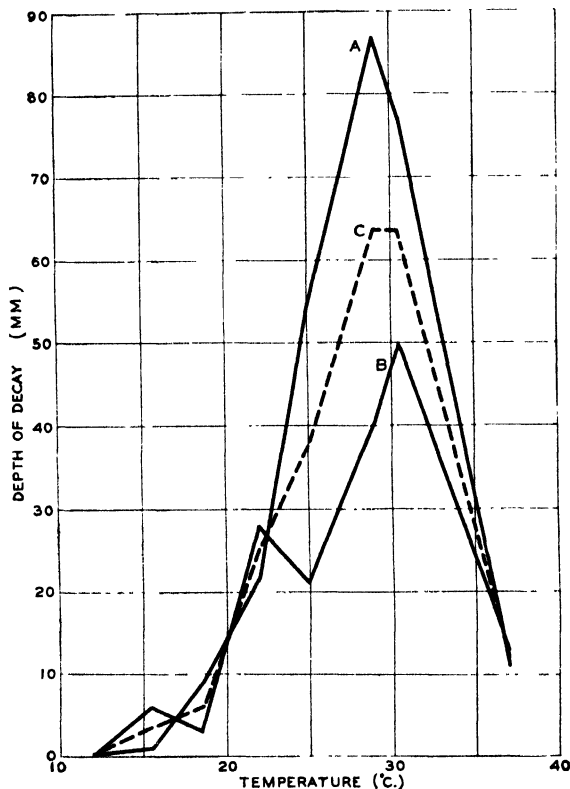


FIGURE 2—Depth of decay in sweetpotatoes caused by *Sclerotium bataticola* at various temperatures. A, Roots inoculated when freshly wounded; B, wounded roots stored for 10 days at 13° C. and at 59 and 69 percent relative humidity before inoculation, C, average of A and B.

roots inoculated when freshly wounded, the maximum amount of decay during 10 to 12 days' storage at various temperatures occurred at 29° (table 19 and fig. 2, A). At temperatures above or below 29°, the rate of decay declined rapidly. A similar curve was obtained in connection with roots subjected to an initial storage at 13° and 59 and 69 percent relative humidity before inoculation and a subsequent storage at various temperatures, except that the maximum amount of decay occurred at 30° to 31° instead of at 29° and that the amount of decay was less at most of the temperatures (table 19 and fig. 2, B). Curve C in figure 2 was plotted from the average depth of decay at a given

temperature in the roots subjected to the two types of treatment. The curve is slightly smoother and the optimum lies between 29° and 30° to 31°. It will be remembered that the optimum temperature for growth of *S. bataticola* is 31°. Unfortunately, a temperature between 31° and 37° was not employed in either the infection or growth experiments; had one been used it is possible that the optimum temperature for growth might have been shifted to a slightly higher temperature. The fact that the rate of decay in some instances has been greater at 29°, and even at 28° (table 18) in one experi

at 31°, would seem to indicate that the optimum temperature must lie between 28° and 31°.

TEMPERATURE LIMITS OF INFECTION

As has been noted in the results already discussed, the rate of infection and decay at a temperature of 12° C. is very slow. The results given in table 20 show this fact more clearly. The data were obtained from the roots stored at four relative humidities at 12° and at 15.5° and 17.5° in one of the experiments discussed in connection with table 19. The length of storage at these temperatures was 44 days, after which the roots from all the conditions of storage were stored for 16 days at 21° and 89 percent relative humidity. Not only was infection slow in taking place at all the conditions of storage but the advance of decay was relatively slight. In many instances *Sclerotium bataticola* was displaced by other organisms, especially forms of *Fusarium*. These results indicate that 12° is near the lower limit for infection and decay. Incidentally, it will be noted that the roots initially stored at 26° and 92 percent relative humidity before inoculation continued to be practically free from infection. These roots likewise remained practically free from infection by such organisms as *Fusarium*, etc., which often displace *S. bataticola* at a temperature of 12°.

TABLE 20.—*Relation of duration of storage to infection of sweetpotatoes (Yellow Jersey variety) by Sclerotium bataticola at certain temperatures and relative humidities*

Storage conditions subsequent to inoculation		Relation of indicated storage period to infection and decay in roots inoculated—												Following wounding and 10 days' storage at 26° C. and 92 percent relative humidity ¹							
		Immediately following wounding						Following wounding and 10 days' storage at 13° C. and 57 percent relative humidity													
		12 days		20 days		44 days ²		60 days		12 days		20 days		44 days ¹		60 days		44 days ¹		60 days	
Temperature (° C.)	Relative humidity	Depth of decay		Infection		Depth of decay		Infection		Depth of decay		Infection		Depth of decay		Infection		Depth of decay		Infection	
		Per-cent	Mm	Per-cent	Mm	Per-cent	Mm	Per-cent	Mm	Per-cent	Mm	Per-cent	Mm	Per-cent	Mm	Per-cent	Mm	Per-cent	Mm	Per-cent	Mm
17.5.....	88	20	1	20	1.0	40	3.6	100	5.5	100	2.4	100	5.0	100	5	100	5	100	0	0	
15.5.....	92	0	0	40	1.6	100	4.5	100	4.6	80	4.6	100	8.8	100	31	100	31	100	0	0	
12.0.....	93	0	0	20	0.8	100	6.7	100	0	0	0	33	1.0	100	3	100	3	100	0	0	
2.0.....	85	0	0	0	0	100	2.4	100	0	0	0	20	1.4	100	9	100	16	100	0	0	
2.0.....	76	0	0	0	0	80	4	100	4	0	0	0	0	100	3	100	6.3	100	0	0	
2.0.....	63	0	0	100	4.6	100	7	100	0	0	0	0	0	80	2.4	100	3.8	100	20	20	

¹ There was no infection after storage periods of 12 or 20 days² During the interval between 44 and 60 days all roots were stored at 21° C. and 89 percent relative humidity

The upper limit for infection of sweetpotatoes by *Sclerotium bataticola* has not been accurately determined, but judged by the rate of decay at 37° C. (table 19) and by the slowness of growth at 40° and 42° (table 17), 37° approaches this limit.

EFFECT OF DURATION OF INITIAL STORAGE OF WOUNDED SWEETPOTATOES ON INFECTION BY *SCLEROTIUM BATATICOLA* DURING SUBSEQUENT STORAGE

In one experiment, 5 wounded roots (cross section tip) of the Yellow Jersey variety were stored at 29° C. and 96 percent relative humidity for intervals of 1 to 10 days, inclusive. At the end of 10 days all these roots, including 5 freshly wounded ones, were inoculated with *Sclerotium bataticola* by dipping the cut end into a mass of corn meal mixed with the fungus and were stored at 28° and 89 percent relative humidity for 28 days. One hundred percent of the roots inoculated when freshly wounded became infected, 80 percent of those inoculated after 1 day's storage, 40 percent of those inoculated after 2 days' storage, and 20 percent of these inoculated after 3 days' storage. None of the roots inoculated after being stored for more than 3 days became infected.

Another experiment, run with the Yellow Jersey and Nancy Hall varieties, was similar to the foregoing in all respects, except that (1) the initial and subsequent storage temperature and relative humidity were 25.5° and 94 percent, respectively, and (2) 2-day intervals between inoculations instead of 1-day intervals were used in the case of the Yellow Jersey variety. By the end of 24 days, all the Yellow Jersey roots inoculated when freshly wounded were infected and 60 percent of those stored 2 days before inoculation were infected, but none of the roots stored for more than 2 days before inoculation showed any infection. Eighty percent of the Nancy Hall roots inoculated when freshly wounded, 80 percent of those inoculated after 1 day's storage, and 100 percent of those inoculated after 2 days' storage became infected. There was no infection in any of the roots inoculated after initial storage periods longer than 2 days.

In a third experiment (table 21) cured Yellow Jersey roots were wounded in the usual fashion (cross section tip), stored at temperatures of 22°, 25°, 29°, and 31° C., respectively, combined with high humidity, for periods of 1, 2, 3, 4, and 5 days. Then they, with a check freshly wounded, were inoculated with *Sclerotium bataticola* and stored for 16 days at 25° and 94 percent relative humidity, and for an additional period of 24 days at 21° and 91 percent relative humidity.

No infection occurred in roots stored for more than 3 days at 31° C., more than 2 days at 29°, more than 2 days at 25°, or more than 4 days at 22°. Some variation in these limits of initial storage might be expected with a variation in the subsequent storage conditions. In other words, if the process of healing is not complete by the end of the initial storage period, infection may take place during subsequent storage that is sufficiently unfavorable to the healing process and sufficiently favorable to the activities of the pathogene. If the process of healing is complete by the end of the initial storage period, subsequent storage has little effect on infection.

TABLE 21.—Effect of preliminary storage of wounded sweetpotatoes for periods of from 0 (check) to 5 days before inoculation on infection by *Sclerotium bataticola* during subsequent storage

Storage conditions between time of wounding and inoculation and number of roots used				Infection and decay after inoculation and storage for periods indicated ¹			
Temperature (° C)	Relative humidity	Interval	Roots used	16 days		40 days	
				Roots infected	Total depth of decay	Roots infected	Total depth of decay
	Percent	Days	Number	Percent	Mm	Percent	Mm
31	94	0 (check)	10	80	47	80	336
31	94	1	5	5	20	10	18
31	94	2	5	0	0	0	0
31	94	3	5	20	3	20	8
31	94	4	5	0	0	0	0
31	94	5	5	0	0	0	0
29	97	1	5	20	5	20	3
29	97	2	5	0	0	20	4
29	97	3	5	0	0	0	0
29	97	4	5	0	0	0	0
29	97	5	5	0	0	0	0
25	95	1	5	20	3	20	3
25	95	2	5	40	23	40	34
25	95	3	5	0	0	0	0
25	95	4	5	0	0	0	0
25	95	5	5	0	0	0	0
22	93	1	5	40	91	40	145
22	93	2	5	20	5	40	33
22	93	3	5	40	95	40	182
22	93	4	5	20	4	20	3
22	93	5	5	0	0	0	0

¹ For the first 16 days storage was at 25° C and 94 percent relative humidity, for the last 24 days at 21° and 91 percent relative humidity.

DISCUSSION AND CONCLUSIONS

Some of the factors influencing infection and decay of sweetpotatoes by the various pathogens discussed herein are as follows: Presence or absence of the pathogene, infection and decay by other fungi, amount of inoculum, wounding—including extent, type, and degree—suberization, periderm formation, temperature, humidity, and time.

Rhizopus tritici and *R. nigricans* (rhizopus soft rot), *Fusarium oxysporum* (surface rot), and species of *Fusarium* (including *F. oxysporum*) causing end rot seem always to be associated with sweetpotatoes in storage, for in the absence of artificial inoculation some infection from these pathogens generally takes place with certain types of wounds and under certain conditions of temperature and humidity. There are indications that *R. tritici* and *R. nigricans* are more abundantly present on the roots as the storage season advances than at harvest time. No information is available relative to the amount of *F. oxysporum* or other forms of *Fusarium* present on sweetpotatoes at different times during the storage season or the effect that a change in the amount present may have on the amount of surface rot and fusarium end rot that develops. It is probable that under certain conditions of storage there is an increase during the storage season in the amount of these fungi present in the storage

house. There are indications that *Diplodia tubericola* and *Sclerotium bataticola* are not always present on sweetpotatoes in storage. This is shown by the fact that infection rarely occurs in sweetpotatoes grown and stored in the vicinity of Washington, D. C., although they have often been stored under conditions favorable for the development of the rots produced by these pathogens. However, these fungi are fairly common in the southern portion of the sweetpotato areas, and in some localities are associated rather consistently with sweetpotatoes in storage, and decay will develop when the handling of the roots and the conditions of storage are favorable.

Openings of some kind, such as wounds, broken rootlets, lesions produced by other fungi, etc., in the skin of sweetpotatoes, through which the pathogens (*Rhizopus tritici*, *R. nigricans*, *Fusarium oxysporum*, other species of *Fusarium*, *Diplodia tubericola*, and *Sclerotium bataticola*) of the various diseases discussed herein may enter, seem to be an essential condition of infection in the case of each pathogene. Considerable indirect evidence obtained from time to time shows fairly conclusively that these fungi cannot penetrate the unbroken skin of the sweetpotato. This evidence is essentially that (1) infection is consistently associated with wounding and (2) that infection is consistently absent in roots in which the unbroken skin has been exposed to the action of the pathogens for various periods in the inoculation experiments and throughout the storage season in some of the storage experiments.

Some, and probably all, of these fungi are able to penetrate sweetpotatoes through lesions produced by other fungi. The primary invader may be one of the foregoing fungi, or it may be a fungus that is able to penetrate unwounded sweetpotato tissue under special conditions (10). Forms of *Fusarium* have been shown to commonly invade lesions of *Mucor racemosus*, *Alternaria*, *Botrytis*, species of *Rhizopus*, and lesions of mottled necrosis. *Diplodia tubericola* and *Sclerotium bataticola* also have been observed to invade lesions produced by some of these fungi.

Injury such as that produced by freezing temperature or by a temperature of 40° C. and above may result in an invasion of some of these fungi when the roots are again restored to more normal temperatures.

In the presence of the various pathogens, mechanical wounding is the chief predisposing condition leading to infection of sweetpotatoes in storage. The wounding chiefly responsible for decay of sweetpotatoes in storage and in transit results from harvesting and storing operations, handling in storage and in preparation for the market, gnawing by rodents in the storage house, and mishandling of railroad cars in transit. With an increase in the intensity and extent of wounding there is an increase in the percentage of infection by *Rhizopus tritici* and *R. nigricans*. The relation of degree and extent of wounding to infection by other pathogens has not been studied. The character of the wounding is a factor in determining the occurrence of rhizopus soft rot, surface rot, and fusarium end rot. Infection by *R. tritici* and *R. nigricans* takes place more readily in bruised than in cut or in sharply broken tissues. These pathogens rarely invade sweetpotatoes through skinned areas, and the percentage of infection is small in roots in which the tip ends have been cut or broken off.

The absence of *Rhizopus* infection in skinned areas enables *Fusarium oxysporum* to establish itself whenever storage conditions unfavorable to suberization and periderm formation are sufficiently prolonged to permit infection. Likewise the absence of *Rhizopus* infection in roots in which the tip ends are cut or broken off enables species of *Fusarium* or other organisms under certain conditions of storage to invade the wounded tissues and thus produce end rot. Invasion by species of *Fusarium* and *Penicillium* occurs in wounded tissues of any kind when the conditions of storage immediately after wounding are unfavorable for healing, the duration of storage is sufficiently long, and the wounds are not invaded by species of *Rhizopus*, *Diplodia tubericola*, or *Sclerotium bataticola*.

The presence of a suberized layer in wounded tissue at times seems to inhibit infection by various pathogenes, and in some instances may prevent infection, although this fact has not been clearly established. Since the formation of a suberized layer is an essential preliminary to periderm formation, its power of inhibition in some instances probably is a decisive factor in delaying infection until the more effective barrier (the periderm) is developed. A periderm coextensive with the wounded area forms an almost, if not quite, perfect barrier against infection by any of the foregoing pathogenes.

Infection of wounded sweetpotatoes by the various pathogenes sometimes occurs during storage subsequent to a 10 days' initial storage at temperatures of 12° to 19° C. combined with high humidity. Suberization occurs at all these temperatures (12° to 19°) within 10 days. The presence of such infection during subsequent storage indicates that either suberization is not coextensive with the wounded tissue by the end of the initial 10-day storage or that it is not an absolute barrier to infection. The earliest that periderm formation has been observed to occur at 12° is 25 days; in one instance it occurred within 11 days at 14.7°, but in another it was absent after 10 days at 15.7°, and 11 days was required for its formation at 19°. The time required for periderm formation also varied somewhat with the lot of roots used, and even if it was found present in a given case it may not always have been coextensive with the wounds. The histological study was confined to limited samples that were representative of the tissues involved but were never coextensive with the wounded tissues concerned in the infection experiments. It will be seen from these considerations that 10-day storage at temperatures of 12° to 19° probably does not always insure complete healing. In the absence of healing during the initial storage period some infection may occur or periderm formation may go on to completion, depending upon the temperature and humidity of the subsequent storage.

Temperature, in addition to being an indirect factor in infection and decay of sweetpotatoes because of its influence on suberization and periderm formation, is a direct factor in determining the limits of infection and in its effect on the rate of infection and decay.

That either free moisture or high atmospheric humidity is favorable to the growth and infection activities of the various pathogenes is shown by the fact that they grow well on carrot agar and readily decay the moist tissue of sweetpotatoes. It is believed that air moisture may become a limiting factor in the growth and infection activities of these fungi if reduced beyond a certain atmospheric content. This problem has not been the object of special study except in the case of infection by *Rhizopus* under certain conditions.

At a temperature of 23° C. infection in halved roots is limited at relative humidities below 75 percent and almost excluded at relative humidities of 48 to 50 percent, because of the effect of this reduction in moisture upon the pathogenes. The effects of relative humidity on these fungi at temperatures of 12° and 28° are not so definitely known. Atmospheric humidity is a more important factor as it indirectly affects infection by all the storage rot fungi under consideration through its effect on suberization and periderm formation than as it affects these fungi directly. Not only is this healing process limited to certain relative humidities, but its rate is increased as the relative humidity is raised above the lower limit of suberization and periderm formation.

The time required for the initiation of decay by the various pathogenes varies with the pathogene, the temperature, and the humidity of the storage chamber. This period also may be affected by the rate of suberization and periderm formation, which also varies with the temperature and humidity of the storage chamber. Whether the period during which wounded sweetpotato roots are exposed to a given storage condition is a limiting factor in infection depends on its duration and on the disease, the amount of inoculum present, the type of wounding, and the subsequent storage conditions.

SUMMARY

During storage the infection of sweetpotatoes by the various storage-rot fungi is influenced by the occurrence of these pathogenes. *Rhizopus nigricans*, *R. tritici*, *Fusarium oxysporum*, and species of *Fusarium* (including *F. oxysporum*) that cause end rot are generally found associated with sweetpotatoes in storage, whereas the occurrence of *Diplodia tubericola* and *Sclerotium bataticola* is more regional, being more commonly limited to the southern portion of the sweetpotato area.

Injury of some kind, such as is produced by wounds, by extremes of temperature, or by other fungi, seems to be an essential preliminary to infection by all the foregoing pathogenes. Mechanical wounding is by far the most important type of injury leading to infection.

Infection of sweetpotatoes by the various pathogenes is influenced by the extent, degree, and kind of wounding. *Rhizopus* infection is favored by bruising and extended wounding, and rarely occurs in skinned areas or in roots with tip ends sharply broken or cut off. Surface rot (*Fusarium oxysporum*) occurs in skinned areas, through injured sprouts or in tissue beneath the sprouts that has been wounded by breaking them off, and through broken secondary rootlets in uncured roots. Species of *Fusarium* invade wounded tissues in roots stored at conditions of temperature and humidity that prevent or greatly retard wound-cork formation. A large percentage of the roots with tip ends cut or broken off and stored under such conditions become infected with *Fusarium*.

A suberin layer seems to inhibit and may sometimes prevent infection by the various fungi.

A wound-periderm layer is an effective barrier against infection by various storage fungi and retards the loss of moisture and consequent shriveling.

Judged from the infection data as well as the histological study, the rate of wound healing tends to increase as the temperature (combined with relative humidities above 90 percent) is raised from 12° to

about 32° C., when it commences to decline. Twenty-five days was required for periderm formation at 12°.

If the moisture content of the air of the storage chamber is reduced beyond a particular point at each of the temperatures 12°, 23°, and 28° to 29° C., healing of wounds in sweetpotatoes will not occur. As the moisture content of the air is raised above this point the rate of healing tends to increase. The range of relative humidities at 12° at which healing occurs is narrow (between 90 and 100 percent). As the temperature is raised to 23° and 28° to 29° the range of relative humidities at which healing takes place becomes progressively wider, the extent of the range depending somewhat on the duration of storage.

Temperature as it affects the fungi producing the various rots is a factor in determining the rate and limits of infection. Although rate of infection increases as the temperature is raised above a minimum, the data show that the increase in rate is not as marked as in the case of wound healing.

There are indications in certain instances that as the moisture content of the air of the storage room is reduced beyond a certain point it tends to limit infection by its action on the pathogenes.

The optimum, minimum, and maximum temperatures for growth of *Diplodia tubericola* are approximately 29° to 31°, 8° to 13°, and 37° to 40° C., respectively. Infection of sweetpotatoes by *D. tubericola* has been obtained at temperatures from 12° to 37°, the most rapid decay occurring at 29° to 31°. The optimum, minimum, and maximum temperatures for growth of *Sclerotium bataticola* are approximately 31°, 8°, and 42° C., respectively. Infection of sweetpotatoes by *S. bataticola* has been obtained at temperatures from 12° to 37°, the most rapid decay occurring at 29° to 31°.

The data presented show that if sweetpotatoes are subjected to 10 days of curing at temperatures from 22° to 32° C., combined with relative humidities of 90 percent and above, protection is developed in wounded tissue against the loss of moisture and consequent shriveling and against infection by *Rhizopus tritici*, *R. nigricans*, *Fusarium oxysporum*, species of *Fusarium* causing end rot, *Diplodia tubericola*, and *Sclerotium bataticola*. These and other data show that this protection is more effectively maintained in storage at 10° to 15° C. if the relative humidity, after curing, is above 80 percent.

If wounded sweetpotatoes are stored 10 days at temperatures from 12° to 37° C., combined with relative humidities above 90 percent, before inoculation in the case of *Diplodia tubericola* and *Sclerotium bataticola* and without inoculation in the case of *Rhizopus tritici*, *R. nigricans*, *Fusarium oxysporum*, and species of *Fusarium* causing end rot, infection during subsequent storage at different conditions is usually limited to roots stored at initial temperatures of 12° to 19° C. (sometimes as high as 21°), increasing with a lowering of the temperature. Since storage for 10 days at initial temperatures between 21° and 32° (combined with high humidity) permitted the wounded tissue to heal, these combinations gave almost complete protection against infection during subsequent storage at various conditions of temperature and relative humidity. As the initial temperatures were raised above 32° there was sometimes greater infection during subsequent storage than in roots initially stored at 21° to 32°.

Five days of initial storage of wounded sweetpotatoes at 25° C. and 94 percent relative humidity and 3 days at 29° and 96 percent

relative humidity, before inoculation, were required for the roots to develop sufficient wound periderm to prevent infection by *Diplodia tubericola* during subsequent storage. In the case of *Sclerotium bataticola*, the length of initial storage required to prevent infection during subsequent storage was 4 days at 31° and 94 percent relative humidity, and at 29° and 96 to 97 percent relative humidity, 3 days at 25° and 95 percent relative humidity, and 5 days at 22° and 94 percent relative humidity.

Infection by *Diplodia tubericola* and *Sclerotium bataticola* took place as readily in roots wounded and stored for 10 days at 13° C. and relative humidities of 59 and 69 percent before inoculation as in roots inoculated when freshly wounded, showing that healing had not taken place under these conditions. A 10-day initial storage of wounded roots, before inoculation, at 26° and 29°, combined with 92 and 97 percent relative humidity, respectively, prevented infection during subsequent storage, showing that these initial storage conditions were favorable to wound healing.

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VARIATION AND CORRELATION IN GRAIN YIELD AMONG 1,500 WHEAT NURSERY PLOTS¹

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INTRODUCTION

In the future, increased grain yields of wheat probably will be secured by smaller and smaller increments, and the agronomist will have to use a finer, more accurate "sieve" in order to isolate the more productive wheats. This paper analyzes the variation and correlation in grain yield among 1,500 wheat nursery plots. Certain of the facts outlined will, the writer believes, permit the estimation of a smaller experimental error and show why some seeding arrangements give better results than others.

In the past many experiments have been conducted to study the variability of field crops of all kinds and the means of reducing it to a minimum. For this purpose uniformity trials similar to the one about to be considered have generally been used. Comparatively little work has been done, however, toward determining the correlation between adjacent plots and those at a greater distance apart. This paper, therefore, is largely concerned with this part of the problem in relation to the general question of variability. The following investigators have considered various phases of the question: Mercer and Hall (15),³ Hall and Russell (9), Montgomery (16, 17), Kiesselbach (13), Lyon (14), Day (3), Stadler (18, 19), Hayes (12), Forster and Vasey (6), Garber, McIlvaine, and Hoover (7), Stringfield (20), Student (1), Summerby (21), Wood and Stratton (24), Griffec (8), Engledow and Yule (4), Christidis (2), and Harris (10, 11). These are referred to later as they touch upon the subject matter considered.

MATERIAL AND METHODS

The material for this study consisted of 1,500 rows of Federation wheat (C. I. no. 4734),⁴ which is well adapted to the region where it was grown. The seed was pure. The crop was grown in the summer of 1927 on the west end of series 100 on the Aberdeen Substation, Aberdeen, Idaho. The ground had been uniformly cropped the previous year to field peas. The plot was seeded on April 18 with a grain drill that sowed eight rows at a time. The rows were 12 inches apart and 15 feet long. Because the land sloped gently to the south

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³ Reference is made by number (italic) to Literature Cited, p. 356.

⁴ Accession number of the Division of Cereal Crops and Diseases.

and west, levees were necessary at 15- and 30-foot intervals. Figure 1 shows the general arrangement and the system of identifying individual series and rows. During the growing season the crop was

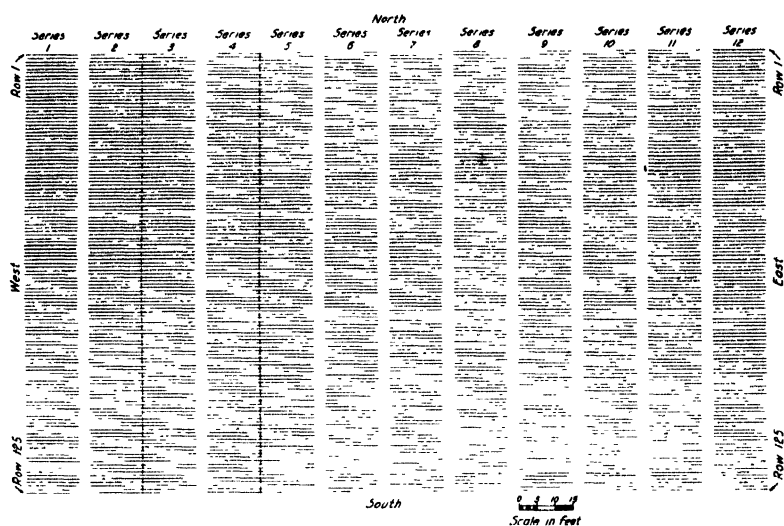


FIGURE 1.—Field plan of uniformity experiments, showing general arrangement of rows. Series 2 and 3, 4 and 5, were grown as 30-foot rows but were cut in 15-foot lengths at harvest time.

irrigated four times. The average annual rainfall at Aberdeen is about 7 inches. The individual rows were harvested in August and

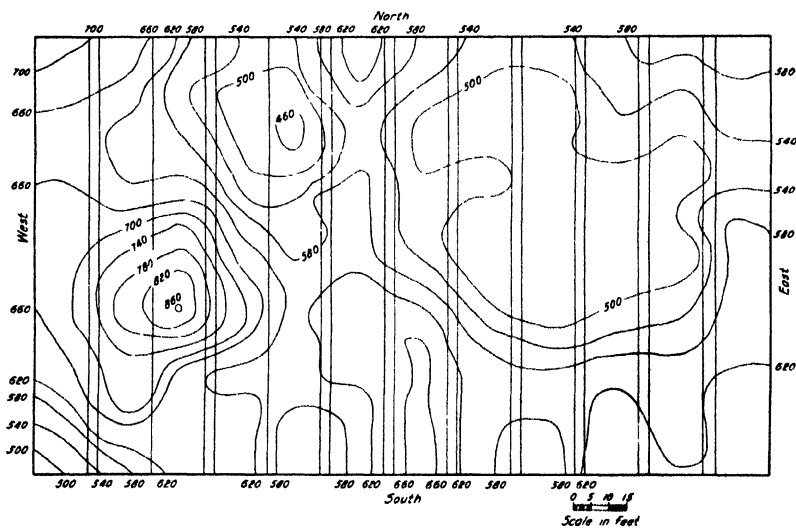


FIGURE 2.—Contour map of the grain yields obtained. The lines are at 40-g intervals.

threshed with a small nursery thresher. The grain yields, recorded in grams per row, are shown in table 1. A contour map of the yields obtained in the plot is shown in figure 2.

The wheat yielded 63 bushels per acre, which is above the average for that region. No disease or insect damage was noticed. Although the height of plants and the weight of straw per row were recorded, only the yield of grain is discussed in this paper.

TABLE 1.—Yield of grain from each of 1,500 15-foot rows of wheat, 12 inches apart, grouped in 12 series of 125 rows each

Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Series 7	Series 8	Series 9	Series 10	Series 11	Series 12
715	595	580	580	615	610	540	515	557	665	560	612
770	710	655	675	700	690	565	585	550	574	511	618
760	715	690	697	655	725	665	647	665	705	644	705
695	615	685	555	585	630	550	527	553	616	573	570
755	730	670	580	545	627	580	525	495	565	599	612
745	670	585	560	550	710	590	545	538	587	630	604
645	690	550	520	450	630	535	505	530	535	517	553
585	495	455	470	445	555	590	457	427	461	531	559
560	540	460	500	505	595	545	530	498	538	453	600
685	730	610	500	555	605	535	534	503	616	638	630
755	810	665	570	525	715	650	550	613	607	742	657
640	635	585	465	430	615	550	515	369	493	635	567
725	655	530	455	465	560	550	460	455	504	519	555
715	775	615	545	530	685	595	510	507	561	581	537
700	705	555	440	455	639	555	425	476	648	532	552
640	655	495	435	485	585	475	405	422	516	458	559
620	635	495	445	455	627	505	465	419	591	545	537
750	630	555	455	510	575	530	470	427	545	572	582
700	675	610	540	530	675	580	460	513	599	595	675
630	645	490	445	440	585	490	427	400	542	474	563
625	540	495	415	445	539	440	439	455	485	461	533
670	670	560	485	445	507	555	450	516	570	553	623
655	630	540	465	460	569	465	435	466	454	458	593
570	535	525	455	415	510	460	420	393	482	423	446
575	495	510	480	395	555	440	450	437	541	525	514
600	645	595	515	470	550	515	445	508	598	517	533
730	730	710	565	470	565	440	557	490	591	594	597
620	635	595	485	435	530	490	470	465	521	454	537
627	600	530	455	425	515	395	455	462	484	595	578
665	685	685	527	480	525	550	465	497	596	563	634
627	605	510	440	450	500	470	415	510	531	478	514
615	525	610	515	395	510	455	539	485	554	448	513
595	625	600	449	435	510	515	525	590	522	496	514
649	580	630	485	480	610	525	515	574	525	458	518
695	650	685	550	515	685	570	525	613	560	532	525
630	610	545	465	460	555	500	575	438	477	480	507
595	555	580	505	465	540	425	490	467	479	444	577
615	635	660	550	525	645	515	520	477	510	465	533
705	660	595	495	465	595	450	505	417	489	389	533
575	530	600	460	410	500	460	505	459	431	438	578
530	555	570	450	425	490	450	495	426	443	449	469
715	680	685	505	535	600	495	480	474	401	495	533
795	715	740	560	585	585	535	590	518	513	541	548
660	635	620	495	510	585	520	475	444	405	579	469
615	580	595	490	505	555	450	470	498	442	474	484
690	645	630	690	565	645	530	550	454	474	592	548
675	615	640	505	550	615	535	570	465	456	513	563
770	625	685	570	575	685	540	475	534	456	467	548
695	700	770	540	515	685	530	495	508	454	422	600
690	660	705	570	545	645	500	540	494	500	552	687
720	730	825	665	605	665	520	550	584	546	537	630
670	640	710	500	535	575	495	455	512	497	405	600
655	590	675	575	515	570	490	470	460	427	472	589
780	790	855	625	575	655	530	549	575	552	503	582
750	705	770	575	560	565	510	435	486	470	502	570
585	620	685	620	550	520	435	490	429	485	534	619
665	605	680	570	560	560	445	455	461	463	533	627
735	760	790	645	605	595	560	485	466	487	476	645
795	795	850	700	655	635	540	470	521	582	584	757
645	685	720	610	520	559	460	440	425	485	462	608
630	695	665	630	510	575	445	439	433	423	450	627
685	840	875	640	575	625	570	490	514	539	486	717
695	780	790	575	520	600	545	470	455	486	488	619
705	745	840	710	640	610	500	530	498	479	434	574
655	735	840	640	610	545	560	450	469	562	453	570
640	820	795	660	580	615	585	460	461	472	504	690
730	765	890	770	690	660	575	490	485	475	539	582
670	690	785	645	510	560	535	405	474	455	458	555
685	790	770	665	600	545	555	390	380	461	439	615
660	825	960	750	660	680	620	510	501	490	534	694
705	805	860	635	540	650	555	435	479	457	451	649
610	720	705	615	540	625	595	380	379	483	502	608
640	735	805	665	535	605	580	430	427	441	498	638
690	855	905	700	615	650	615	495	531	448	453	645
715	765	945	820	695	750	685	530	511	580	510	762

TABLE 1.—Yield of grain from each of 1,500 15-foot rows of wheat, 12 inches apart, grouped in 12 series of 125 rows each—Continued

Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Series 7	Series 8	Series 9	Series 10	Series 11	Series 12
685	750	825	715	580	660	655	505	414	451	542	642
685	790	790	695	595	625	510	525	425	471	478	514
775	845	905	820	655	675	640	545	474	513	599	597
645	870	890	740	615	655	555	490	433	588	590	567
725	825	920	640	600	690	575	535	451	494	510	567
650	865	935	690	645	640	530	520	476	507	525	563
695	860	960	725	615	710	660	525	438	539	646	623
735	910	975	775	680	700	770	635	506	526	514	634
645	745	815	700	605	615	605	550	455	504	503	589
630	810	730	635	535	650	735	535	458	554	535	615
690	745	840	730	645	650	775	590	557	561	665	619
620	730	775	680	610	610	680	515	537	535	553	570
620	745	660	565	520	635	610	450	476	550	562	575
580	675	690	635	525	605	635	515	464	516	558	559
625	700	725	645	645	580	615	640	516	618	615	604
790	765	725	615	640	705	590	619	527	620	620	645
685	785	655	600	570	615	670	575	506	622	530	600
625	550	590	590	605	505	560	595	629	629	571	698
745	790	675	600	625	685	725	695	686	608	568	672
680	670	630	640	645	650	695	610	627	579	537	675
655	730	615	650	640	645	655	620	611	642	685	630
625	700	675	720	695	680	720	615	595	556	648	578
670	735	645	620	705	695	650	580	623	541	580	630
670	827	685	665	715	715	790	660	658	689	725	675
555	670	590	580	550	665	640	605	553	580	605	634
650	685	505	525	595	590	560	575	592	559	584	518
625	760	625	545	635	635	670	625	672	656	577	645
620	740	575	565	595	610	650	595	515	641	533	623
580	650	570	575	450	445	670	645	587	634	534	574
615	705	550	515	530	485	610	570	565	652	518	623
675	690	615	610	560	625	745	540	584	616	505	668
720	820	720	650	590	615	770	645	615	755	625	713
640	780	640	660	605	540	695	580	615	679	540	570
630	800	715	710	605	575	490	485	564	665	605	582
625	830	795	765	610	630	775	630	614	760	730	619
470	685	680	735	560	590	670	610	556	539	498	608
445	555	605	685	540	615	610	525	640	639	594	739
400	570	555	655	475	615	715	595	595	747	671	795
480	495	650	690	535	600	760	655	498	621	578	615
555	660	715	690	635	685	775	665	652	727	689	728
520	530	650	605	480	545	585	610	561	670	622	623
490	475	595	660	475	545	635	550	524	538	626	552
500	610	590	680	555	625	670	669	541	613	583	585
425	560	570	570	455	605	605	530	544	645	612	537
535	550	540	630	480	565	745	515	467	606	563	510
560	590	560	575	500	475	610	575	492	591	532	439
530	610	600	620	490	585	657	570	577	612	654	630
570	545	635	765	550	675	765	620	674	705	677	660
505	500	580	655	470	565	570	555	537	585	549	619
465	430	510	680	460	600	670	615	629	594	616	784

Five aspects of variation and correlation as a general problem are considered here: (1) The general variability of the field, considered under an extended and a closed system; (2) the correlation between rows at different distances apart; (3) the intraclass correlation and variation within combination plots compounded by different systems of replication; (4) the variation between combination plots compounded by different systems of replication; (5) the principle of maximum contiguity and its practical application.

EXPERIMENTAL RESULTS

GENERAL VARIABILITY UNDER AN EXTENDED AND A CLOSED SYSTEM

In discussing general variability in yield, one must clearly distinguish between the extended system and the closed system.

In the present discussion an extended system is one in which, at each successive consideration of variability, more or less land is used. For example, if the variation of the 125 rows in series 1 is found, and the 125 rows of series 2 are added, making a total of 250 rows (each 15 feet long), and the variation is again computed, then the general variability is investigated under an extended system. One may

increase or decrease the number of units of the same size, or one may keep their number constant and vary their size. In the present case both systems were used but always going from a smaller area of land to a larger one.

In the closed system, on the other hand, the area of land remains the same, but the size and number of plots change. Under this system the variability of numerous smaller plots is studied first, and then a few larger plots are considered. In this study the increase in plot size may conveniently be limited to an increase in length of row. The size might also be increased by adding together rows lying side by side. The effect of shape of plot is discussed later.

The problems to be determined in such a study of plot variability are: (1) The variation when more and more land is added to the experimental area while the size and shape of the ultimate plot remain the same; (2) the effect when more and more land is added and the number of plots remains the same but their size is automatically increased; and (3) the variation when the entire area is used at each consideration but the plots differ in size.

Many studies have already dealt with plot variability, but the literature does not often state under which of the two systems the work was done.

To determine the general variability under the systems outlined above, the mean, the standard deviation, and the coefficient of variation were calculated for each of the 12 series. These figures are given in table 2. The coefficients of variation ranged from 9.98 for series 6 to 18.14 for series 3. The contour map (fig. 2) shows why this is true. Series 3 includes an area of relatively high yields, such as series 6 does not contain. More contour lines cross series 3 than series 6, and this map roughly indicates the variability existing at different points.

TABLE 2.—Mean, standard deviation, and coefficient of variation of grain yield for individual series of nursery plots of wheat

[Each series included 125 rows 15 feet in length]

Series no.	Mean yield per row	Standard deviation	Coefficient of variation
	<i>Grams</i>	<i>Grams</i>	
1	644.58±17.29	81.47±15.15	12.64±0.81
2	682.98±8.96	100.23±6.34	14.68±.95
3	673.38±10.93	122.18±7.73	18.14±1.18
4	590.26±8.51	95.18±6.02	15.96±1.03
5	544.74±6.92	77.42±4.90	14.21±.92
6	609.38±5.44	60.84±3.85	9.98±.64
7	581.22±8.28	92.57±5.85	15.93±1.03
8	524.26±6.11	68.32±4.32	13.03±.84
9	510.18±6.21	69.46±4.39	13.62±.88
10	546.66±6.83	76.34±4.83	13.96±.90
11	541.22±6.48	72.40±4.58	13.38±.86
12	599.78±6.07	67.91±4.30	11.32±.73

¹ Standard error.

Table 3 gives the mean, standard deviation, and coefficient of variation, as determined under the extended system, where the plot size remains the same but successive increments of land are added. The upper section of table 3 shows the variation existing among the 125 rows of series 1 at the west side of the field and then the change in variation from west to east as the other series (125 rows each) are added one by one. In the lower section of table 3 the same method is followed, from east to west. The coefficients of variation so deter-

mined are shown graphically in figure 3. As indicated by the data, variability tends to increase as more land is brought into the experimental area. Although the lines in figure 3 fluctuate somewhat, the trend is upward. As such increased variability is not expected if the laws of random sampling are fulfilled, evidently the laws of sampling have not been strictly adhered to. The difficulty lies in the correlation existing between nearby rows and the significant differences between most of the means and standard deviations of the 125 rows of the 12 series under investigation. The trend, therefore, should be upward, as is shown by table 2 in conjunction with table 3. To illustrate this point, consider series 1 and 2. Series 1 has a mean of 644.58 g and a coefficient of variation of 12.64 percent. Now, if series 2, which has a mean of 682.98 g and a coefficient of variation of 14.68 percent, is grouped with series 1, the inevitable result is increased variability for the larger area. Changes in variability of increased areas depend on two factors, namely, the mean and variability of the rows to be added in relation to the mean and variability of the rows already in the experiment. Reduced variability of the enlarged experimental area is expected if the rows to be added show less variability than those already under consideration and have a mean reasonably close to the latter, and also if they have an extremely small variability and a mean considerably different from that already in the experiment. On the other hand, increased variability is expected if the rows to be added have a greater variability than those already in the experiment and if their mean is reasonably close to the latter, and also if the rows to be added have less variability than those already in the experiment but a mean markedly different from the latter. Sometimes the mean and sometimes the variability of the areas added is the more important factor in changing the variability of the larger area.

TABLE 3.—Mean, standard deviation, and coefficient of variation of grain yield for different series groupings, from west to east and from east to west

WEST TO EAST				
Series grouped (nos)	Rows ¹	Mean yield per row	Standard deviation	Coefficient of variation
	Number	Grams	Grams	
1.....	125	644.58±7.29	81.47±15.15	12.64±0.81
1, 2.....	250	663.78±5.90	93.33±4.17	14.06±.64
1, 2, 3.....	375	666.98±5.37	103.94±3.80	15.58±.58
1, 2, 3, 4.....	500	649.30±4.76	106.33±3.36	16.38±.53
1, 2, 3, 4, 5.....	625	624.39±4.38	109.51±3.10	17.43±.51
1, 2, 3, 4, 5, 6.....	750	625.22±3.77	103.25±2.67	16.51±.44
1, 2, 3, 4, 5, 6, 7.....	875	618.94±3.48	102.94±2.46	16.63±.41
1, 2, 3, 4, 5, 6, 7, 8.....	1,000	607.10±3.29	104.10±2.33	17.15±.39
1, 2, 3, 4, 5, 6, 7, 8, 9.....	1,125	596.33±3.14	105.34±2.22	17.66±.38
1, 2, 3, 4, 5, 6, 7, 8, 9, 10.....	1,250	591.36±2.94	103.89±2.08	17.57±.36
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11.....	1,375	586.80±2.76	102.45±1.95	17.46±.34
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12.....	1,500	587.89±2.58	100.09±1.83	17.03±.32

EAST TO WEST				
12.....	125	599.78±6.07	67.91±4.30	11.32±0.73
12, 11.....	250	570.50±4.81	76.05±3.40	13.33±.61
12, 11, 10.....	375	562.55±3.97	76.97±2.81	13.68±.51
12, 11, 10, 9.....	500	549.46±3.51	78.51±2.48	14.29±.46
12, 11, 10, 9, 8.....	625	544.42±3.09	77.24±2.18	14.19±.41
12, 11, 10, 9, 8, 7.....	750	550.55±2.96	81.17±2.10	14.74±.39
12, 11, 10, 9, 8, 7, 6.....	875	558.96±2.75	81.24±1.94	14.53±.35
12, 11, 10, 9, 8, 7, 6, 5.....	1,000	557.18±2.56	80.90±1.81	14.52±.33
12, 11, 10, 9, 8, 7, 6, 5, 4.....	1,125	561.52±2.49	83.52±1.76	14.87±.32
12, 11, 10, 9, 8, 7, 6, 5, 4, 3.....	1,250	572.71±2.67	94.32±1.89	16.47±.34
12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2.....	1,375	582.73±2.70	100.04±1.91	17.17±.34
12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.....	1,500	587.89±2.58	100.09±1.83	17.03±.32

¹ Each row was 15 feet in length.

² Standard error.

In table 4, under the extended system of determining general variability, the number of plots is kept constant but their size is increased by lengthening the rows, or, in other words, by adding together the

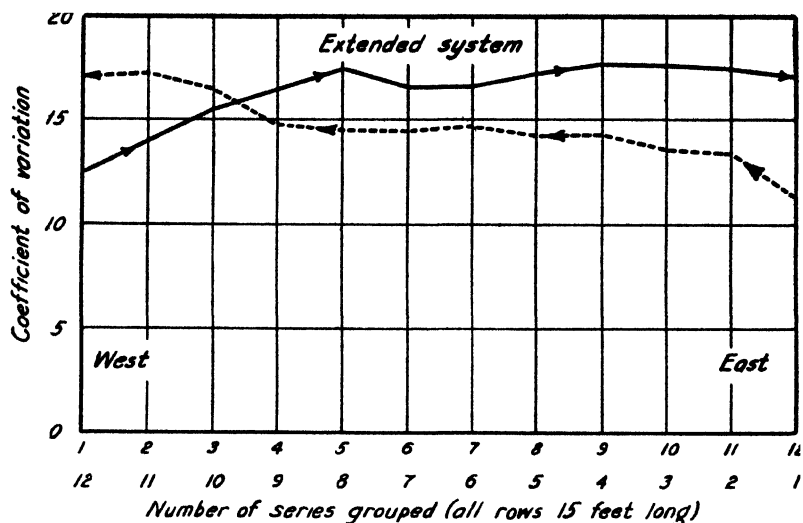


FIGURE 3.—Trend of the coefficient of variation, under an extended system, when different numbers of series are grouped, from west to east (solid line) and from east to west (dotted line)

yields from rows the ends of which were adjacent to each other. The general trend of the coefficient of variation now is downward (fig. 4). In the former extended system, where the size of plot was

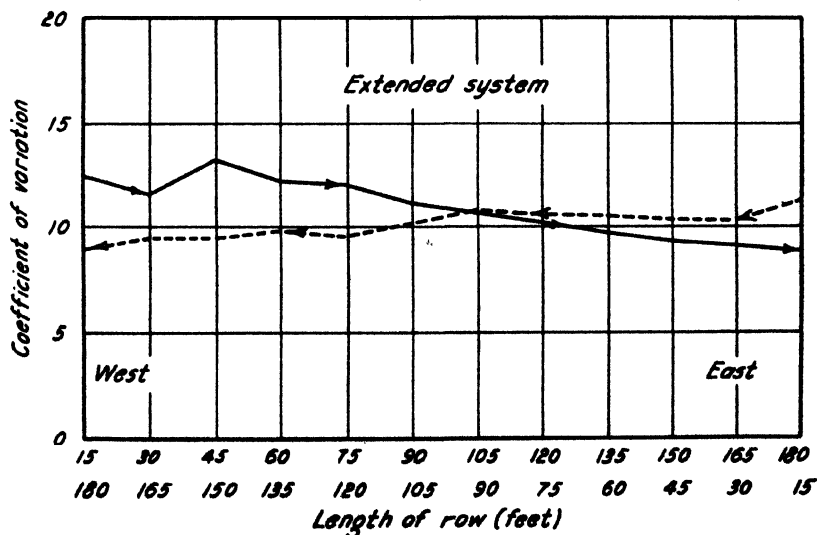


FIGURE 4.—Trend of the coefficient of variation, under an extended system, when the plot size is increased by lengthening the row, from west to east (solid line) and from east to west (dotted line).

kept constant, the variability tended to increase; but in the present case, where the plots are allowed to increase in size while remaining the same in number, variability tends to decrease. This reduction

may be explained on the basis that when the rows are added—for example, row 1 in series 1 and row 1 in series 2—the positive and the negative deviations tend to cancel each other. The variability is not reduced in proportion to the square root of the number of rows combined. It is less than expected, because correlation exists between the rows combined. The sharp increase in the coefficient of variation in table 4 when series 3 was added undoubtedly is a result of the high variability of this series, the coefficient of variation being 18.14 percent (table 2). In most instances the averages of the rows added together were more variable than those of the rows already in the experiment.

TABLE 4.—Mean, standard deviation, and coefficient of variation of grain yield for different series groupings, of 125 rows of various lengths, from west to east and from east to west ¹

WEST TO EAST				
Series grouped (nos.)	Length of row	Mean yield per row	Standard deviation	Coefficient of variation
	Feet	Grams	Grams	
1.....	15	644.58±7.29	81.47±5.15	12.64±0.81
1,2.....	30	1,332.10±14.00	150.57±9.90	11.75±.75
1,2,3.....	45	2,002.60±23.50	262.70±16.61	13.12±.84
1,2,3,4.....	60	2,600.42±28.58	319.55±20.21	12.29±.79
1,2,3,4,5.....	75	3,144.74±34.05	380.69±24.08	12.11±.78
1,2,3,4,5,6.....	90	3,750.50±37.06	414.30±26.20	11.05±.71
1,2,3,4,5,6,7.....	105	4,372.20±42.34	473.33±29.94	10.83±.69
1,2,3,4,5,6,7,8.....	120	4,848.74±44.44	496.80±31.42	10.25±.66
1,2,3,4,5,6,7,8,9.....	135	5,309.08±46.81	523.39±33.10	9.75±.62
1,2,3,4,5,6,7,8,9,10.....	150	5,910.50±49.82	557.04±35.23	9.42±.60
1,2,3,4,5,6,7,8,9,10,11.....	165	6,457.70±53.02	592.79±37.49	9.18±.59
1,2,3,4,5,6,7,8,9,10,11,12.....	180	7,054.50±56.76	634.56±40.13	9.00±.57

EAST TO WEST				
	Length of row	Mean yield per row	Standard deviation	Coefficient of variation
	Feet	Grams	Grams	
12.....	15	599.78±6.07	67.91±4.30	11.32±0.73
12,11.....	30	1,134.98±10.55	117.97±7.46	10.39±.66
12,11,10.....	45	1,682.98±15.68	175.32±11.09	10.42±.67
12,11,10,9.....	60	2,196.26±20.73	231.82±14.66	10.56±.68
12,11,10,9,8.....	75	2,721.38±25.65	286.74±18.14	10.54±.67
12,11,10,9,8,7.....	90	3,303.14±32.20	360.02±22.77	10.90±.70
12,11,10,9,8,7,6.....	105	3,918.50±35.45	396.34±25.07	10.11±.65
12,11,10,9,8,7,6,5.....	120	4,458.02±38.54	430.91±27.25	9.67±.62
12,11,10,9,8,7,6,5,4.....	135	5,057.06±44.94	502.42±31.78	9.93±.63
12,11,10,9,8,7,6,5,4,3.....	150	5,737.70±49.40	552.35±34.93	9.63±.61
12,11,10,9,8,7,6,5,4,3,2.....	165	6,403.82±54.72	611.76±38.69	9.55±.61
12,11,10,9,8,7,6,5,4,3,2,1.....	180	7,054.50±56.76	634.56±40.13	9.00±.57

¹ The rows were lengthened by adding together the yields from rows which had their ends adjacent to each other.

² Standard error.

Under the closed system, a constant area of land is considered but different-sized subunits are used in studying its variability. In each study, furthermore, all the land is used. Table 5 records the data from a treatment of this kind. The size and number of the plots must necessarily change as the study progresses. The change in size in these data has been limited to a lengthening of rows. Figure 5 shows graphically the relationships found. Under the closed system, theoretical curves can be calculated and compared with the actual one. These curves also are shown in figure 5. Only certain row lengths can utilize all the land each time the variability is determined. These lengths are 15, 30, 45, 60, 90, and 180 feet. A gradual reduction in variability occurs as the length of row is increased. For the

15-foot rows it is 17.03 percent, for the 180-foot rows 9.00 percent, of the mean yield. Since increasing the size of plot is equivalent to averaging adjacent plots, this reduction in variability should be proportional to the square root of the number of plots in each group when, and only when, the intraclass correlation coefficient between the plots grouped is zero. The theoretical curve of reduction in variability under these conditions is the lowest one shown in figure 5. It certainly does not agree with the actual results.

Since adjacent rows are positively correlated, as will be shown later, it is interesting to see what form the theoretical curve takes in comparison with the actual when this fact is considered. To compute the

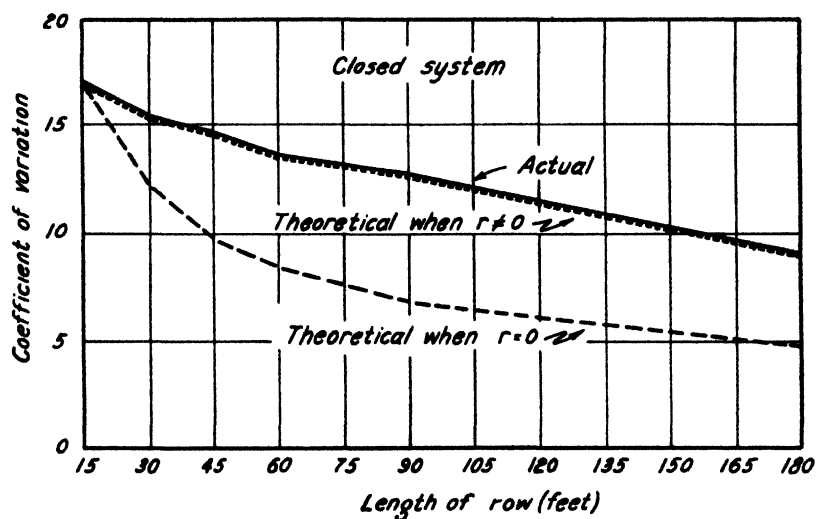


FIGURE 5.—Trend of the coefficient of variation, under a closed system, when rows of different lengths are considered. The theoretical lines when $r=0$ and $r \neq 0$ are also shown.

variability with r as an influencing factor, the following formula given by Yule (25, p. 350) was used:

$$\sigma_m^2 = \frac{\sigma_p^2}{n} [1 + (n-1)r]$$

where σ_m is the standard deviation of the combination plots desired, σ_p , the standard deviation of the ultimate plots, n the number of ultimate plots in the combination plot, and r the intraclass coefficient of correlation for the particular combination plot under consideration. The intraclass coefficients of correlation have been calculated by Harris' method, and the equation is given later. Table 5, column 8, records the intraclass correlations used in ascertaining the theoretical coefficients of variation in column 6. Now, columns 5 and 6 agree—a fact brought out very clearly by figure 5. Change in variability in a closed system of this kind is therefore a function of the number of ultimate plots combined and their intraclass correlation. General disregard of the intraclass correlation unquestionably accounts for the poor agreement between actual and theoretical curves.

TABLE 5.—Mean, standard deviation, coefficient of variation, and intraclass correlation coefficient of grain yield for rows of various lengths, when the entire area is utilized for each row length¹

Length of row (feet)	Rows	Mean yield per row	Standard deviation	Coefficient of variation			Value of intraclass correlation
				Actual	Theoretical when $r \neq 0$	Theoretical when $r = 0$	
	<i>Number</i>	<i>Grams</i>	<i>Grams</i>				
15.....	1,500	587.89±2.58	100.09±1.83	17.03±2.0.32	17.03±2.0.32	17.03±2.0.32	-----
30.....	750	1,175.30±6.55	179.32±4.63	15.25±.40	15.25±.40	12.03±.32	+0.604
45.....	500	1,761.46±11.56	258.64±8.18	14.66±.47	14.66±.47	9.83±.32	+ .611
60.....	375	2,350.18±16.51	319.71±11.68	13.59±.51	13.59±.51	8.51±.31	+ .616
90.....	250	3,526.82±28.71	453.94±20.30	12.86±.58	12.86±.58	6.95±.31	+ .484
180.....	125	7,054.50±56.76	634.56±40.13	9.00±.57	9.00±.57	4.91±.31	+ .214

¹ The rows were lengthened by adding together the yields from rows which had their ends adjacent to each other.

² Standard error.

CORRELATION BETWEEN ROWS AT DIFFERENT DISTANCES APART

In the previous section, the marked effect of the coefficient of intraclass correlation upon the variability between combination plots was pointed out. Student (1) makes the following statement: "The art of designing all experiments lies even more in arranging matters so that r_{ab} (the correlation between paired plots) is as large as possible, than in reducing σ_a^2 and σ_b^2 ." With this in mind, a study was made of the correlation between the ultimate plots when the distance between them was varied.

The method of calculating the correlations is as follows. Suppose the correlation coefficient between rows 8 feet apart is desired. It may be designated r_{19} because the distance between rows numbered 1 and 9 is 8 feet. The correlation table is entered with rows 1 and 9 of series 1 as the first pair, rows 9 and 17 as the second pair, rows 17 and 25 as the third pair, etc. Thus there were 15 pairs for each series, or 180 pairs for the 12. The procedure of arriving at pairs in the other series was the same as for series 1.

As noted above, the rows were sown with a grain drill that seeded eight rows at a time. At harvest time, it was noted that the distance between some of the rows was not exactly 12 inches. The result was a small amount of systematic variation within a drill width. Some variations also occurred in the distance between rows where one drill width of rows bordered another. To avoid these systematic errors, no outside row of any drill width was used as a member of a pair in the present correlation study. Actually, in working out r_{19} , the correlation table was entered with the first pair as rows 3 and 11, the second as 11 and 19, the third as 19 and 27, etc. For r_{14} the first pair was rows 3 and 6, the second 11 and 14, the third 19 and 22, etc. All the coefficients were calculated for rows 15 feet long.

After the correlation scatters had been made they were coded, and r was computed by the following formula:

$$r = \frac{\sigma_1^2 + \sigma_2^2 - \sigma_{1-2}^2}{2(\sigma_1 \sigma_2)}$$

A calculating machine and a short-cut method reduced the labor to a minimum.

The values for r obtained at different distances are recorded in table 6 and are shown graphically in figure 6. Most of the coefficients calculated were on distances that lie in a north-south direction. To see whether an east-west direction would give a different value, the coefficients for 18- and 36-foot distances were calculated. These are

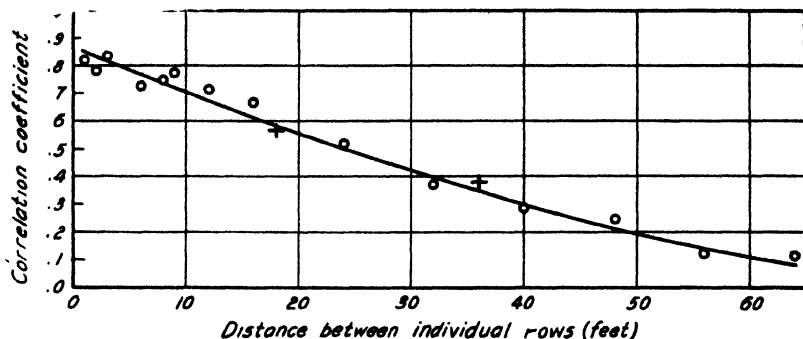


FIGURE 6—Correlation between grain yields of 15-foot rows at different distances apart. The circles (o) indicate correlations when the distance between rows was in a north-south direction, and the crosses (+) when the distance was east-west. The line drawn to fit the points is a "freehand" curve.

shown as crosses (+) in figure 6; the circles (o) are for coefficients in a north-south direction.

TABLE 6. Correlation between grain yield of the ultimate rows (15 feet in length) when the distance separating them is varied

Direction and distance between rows (feet)	Correlation coefficient	Direction and distance between rows (feet)	Correlation coefficient
<i>North to south</i>		<i>North to south</i>	
1	+0.821	32	+0.360
2	+0.781	40	+0.291
4	+0.828	48	+0.246
6	+0.721	56	+0.179
8	+0.750	64	+0.112
9	+0.773	<i>East to west</i>	
12	+0.710	18	+0.569
16	+0.607	36	+0.381
21	+0.516		

¹ Not significantly different from zero.

Figure 6 shows that the correlation falls off as the distance between rows increases. The line drawn to fit the points in this figure is a freehand curve, not one fitted by statistical methods. The correlation decreases at an almost uniform rate as the distance increases. The two east-west values shown as (+) fit very closely to what would be expected on a north-south basis. The values calculated at the 56- and 64-foot distances are +0.179 and +0.112, respectively, but these do not differ significantly from zero. All the other values are significant. The relationship shown here for the value of r and the distance between rows is discussed again later.

INTRAClass CORRELATION AND VARIATION WITHIN COMBINATION PLOTS COMPOUNDED BY DIFFERENT REPLICATION SYSTEMS

The term "replication" is used here in its broadest sense. Forming a combination plot from two adjacent ultimate plots is considered replication just as much as if the plots had been distributed by some random or controlled system and at a greater distance from each other.

For this study the last 5 rows of each series were not used, so that 120 rows remained in each series. This was done because 120, an even number, is exactly divisible by more numbers than is 125. The number of rows used, therefore, was 1,440 instead of 1,500.

The relationships of replication to intraclass correlation and variation within combination plots are considered under two aspects. (1) When the replicates are distributed by contiguous association, one may conveniently speak of size and shape of combination plot in referring to number and arrangement of the contiguous ultimate plots, to whose intraclass correlation and variation they have an important relation. (2) When the replicates are distributed noncontiguously, they may be considered in two ways: (a) Completely at random and (b) at random within certain established limits.

CONTIGUOUS DISTRIBUTION OF REPLICATES

In evaluating the intraclass correlation and variation within combination plots, the intraclass correlation coefficient developed by Harris (10) and the analysis of variance by Fisher (5) were used. The equation for computing the value for the intraclass correlation coefficient r follows:

$$r = \frac{\{[S(C_p^2) - S(p^2)] \div m[n(n-1)]\} - \bar{p}^2}{\sigma_p^2}$$

where

- r = mean correlation between the yields of all the ultimate plots within a combination plot.
- C_p = total yield of a combination plot.
- p = yield of an ultimate plot.
- m = number of combination plots.
- n = number of plots within a combination plot.
- \bar{p} = mean yield of the ultimate plots.
- σ_p = standard deviation of the ultimate plots.
- S = summation.

To illustrate the meaning of r obtained by this method, a combination plot formed from 3 adjacent rows is used. There are then $\frac{n(n-1)}{2}$ or 3 pairs of yields to be entered in the correlation table, namely, yield of row 1 with yield of row 2, 1 with 3, and 2 with 3. If the pairs are entered also as 2 with 1, 3 with 1, and 3 with 2, the correlation table is made symmetrical. This reciprocal method of entry gives $n(n-1)$ pairs of entries for each combination plot, and thus the entries are made for all the other combination plots. Thus, r is the mean correlation coefficient between the yields of all the ultimate plots within a combination plot. A combination plot may be defined as one made by adding together the desired number of ultimate plots. For example, a plot having a width : length ratio of $4/30 = 0.1333$ and a size of 8 rows is formed by adding rows 1 to 4 of series 1 to rows 1 to 4 of series 2. The second plot is made from rows 5 to 8 of series 1 plus rows 5 to 8 of series 2, and so on for the other combination plots.

The intraclass correlation coefficients were calculated for a number of plot sizes and shapes and are given in tables 7 and 8. Table 7 gives the value of r for varying plot sizes when certain definite plot

shapes are considered. In table 8 the value of r is for varying plot shapes when certain definite plot sizes are considered. The data show that the correlation becomes less with an increase in size when any particular shape of plot is considered, and for a constant size becomes less as the plot becomes long and narrow.

TABLE 7.—*Relation of size of plot to intraclass correlation coefficient and to coefficient of variation of yield of grain within and between combination plots compounded by contiguous association, when different shaped plots are considered*

Shape of plot width length	Com- bination plots	Size of plot		Intraclass correlation coefficient	Coefficient of variation	
		Rows	Area		Within combi- nation plots	Between combi- nation plots
<i>Feet</i>	<i>Coeficient</i>	<i>Number</i>	<i>Sq. ft.</i>			
15/15 = 1.0000	96	15	225	+0.660	10.00± ¹ 0.19	14.18± ¹ 1.04
30/30 = 1.0000	24	60	900	+ .469	12.50± .23	12.14± 1.78
8/15 = .5333	180	8	120	+ .681	9.70± .18	14.56± .78
24/45 = .5333	20	72	1,080	+ .477	12.40± .23	12.25± 1.97
4/15 = .2667	360	4	60	+ .710	9.30± .17	15.15± .58
8/30 = .2667	90	16	240	+ .845	11.57± .22	12.99± .98
12/45 = .2667	40	36	540	+ .532	11.74± .22	12.66± 1.44
24/90 = .2667	10	144	2,160	+ .350	13.83± .26	10.77± 2.44
2/15 = .1333	720	2	30	+ .679	9.72± .18	15.72± .42
4/30 = .1333	180	8	120	+ .664	11.32± .21	13.50± 1.72
8/60 = .1333	45	32	480	+ .456	12.66± .24	11.79± 1.26
12/90 = .1333	20	72	1,080	+ .398	13.32± .25	11.21± 1.79
1/15 = .0667	² 1,440	1	15			17.16± .33
2/30 = .0667	360	4	60	+ .558	11.46± .22	13.99± .53
4/60 = .0667	90	16	240	+ .471	12.47± .24	12.18± .92
6/90 = .0667	40	36	540	+ .413	13.15± .25	11.24± 1.27
12/180 = .0667	10	144	2,160	+ .130	16.00± .31	6.68± 1.50
1/30 = .0333	720	2	30	+ .604	10.71± .20	15.26± .41
2/60 = .0333	180	8	120	+ .516	11.93± .23	13.03± .70
3/90 = .0333	80	18	270	+ .439	12.86± .24	11.77± .94
6/180 = .0333	20	72	1,080	+ .143	15.88± .30	6.92± 1.10
1/45 = .0222	480	3	45	+ .611	10.62± .20	14.68± .48
1/60 = .0167	360	4	60	+ .516	11.85± .22	13.60± .52
1/90 = .0111	240	6	90	+ .484	12.23± .23	12.87± .60
1/180 = .0056	120	12	180	+ .209	15.26± .29	9.00± .59

¹ Standard error.

² Number of ultimate plots.

TABLE 8.—*Relation of shape of plot to intraclass correlation coefficient and to coefficient of variation of yield of grain within and between combination plots compounded by contiguous association, when different sized plots are considered*

Size of plot		Shape of plot width length	Combi- nation plots	Intraclass correlation coefficient	Coefficient of variation	
Rows (number)	Area				Within combi- nation plots	Between combi- nation plots
	<i>Square feet</i>	<i>Feet</i>	<i>Coeficient</i>			
2	30	2/15 = 0.1333	720	+0.679	9.72± ¹ 0.18	15.72± ¹ 0.42
2	30	1/30 = .0333	720	+ .604	10.71± .20	15.26± .41
4	60	4/15 = .2667	360	+ .710	9.30± .17	15.15± .58
4	60	2/30 = .0667	360	+ .554	11.46± .22	13.99± .53
4	60	1/60 = .0167	360	+ .516	11.85± .22	13.60± .52
8	120	8/15 = .5333	180	+ .681	9.70± .18	14.56± .78
8	120	4/30 = .1333	180	+ .664	11.32± .21	13.50± .72
8	120	2/60 = .0333	180	+ .516	11.93± .23	13.03± .70
16	240	8/30 = .2667	90	+ .845	11.57± .22	12.99± .98
16	240	4/60 = .0667	90	+ .471	12.47± .24	12.18± .92
36	540	12/45 = .2667	40	+ .532	11.74± .22	12.66± 1.44
36	540	6/90 = .0667	40	+ .413	13.15± .25	11.24± 1.27
72	1,080	24/45 = .5333	20	+ .477	12.40± .23	12.25± 1.97
72	1,080	12/90 = .1333	20	+ .398	13.32± .25	11.21± 1.79
72	1,080	6/180 = .0333	20	+ .143	15.88± .30	6.92± 1.10
144	2,160	24/90 = .2667	10	+ .350	13.83± .26	10.77± 2.44
144	2,160	12/180 = .0667	10	+ .130	16.00± .31	6.68± 1.50
1	15	1/15 = .0667	² 1,440			17.16± .33

¹ Standard error.

² Number of ultimate plots.

The trends in the value of the correlation are those expected on the basis that ultimate plots farther apart are less highly correlated. When two combination plots have the same shape, the ultimate plots of the larger are farther removed from each other than are those of the smaller. And since the value of r is based on all the possible $n(n-1)$ pairs of the ultimate plots compounding a combination plot, a smaller value from the larger combination plot is expected, which is in fact the case. The same reasoning holds true when the combination plot remains constant in size but changes in shape. In the long narrow plot the distance between ultimate plots that go to make up all the pairs is again greater than for a relatively square plot, and hence a lower value for r is to be expected.

All the plot shapes given in tables 7 and 8, when not square, had their greatest length in an east-west direction.

The variability of the ultimate plots within a combination plot is determined by two different methods that give identical results. In one, Harris' intraclass coefficient is used in the equation

$$\sigma_w^2 = \sigma_p^2(1-r)$$

where σ_w is the standard deviation of the ultimate plots within a combination plot; σ_p the standard deviation of all the ultimate plots, secured by dividing the squared deviations by n ; and r the intraclass correlation coefficient. The other method is Fisher's (5) analysis of variance. The principle will not be described here, being found in texts by Fisher (5) and Tippett (23). To illustrate the method, however, the variability within a combination plot of 16 ultimate plots and with a width : length ratio of $8/30=0.2667$ was computed. These data are shown in table 9. The second line of the table refers to that part of the variability which is within combination plots. The standard deviation found was 68.03, or 11.57 percent of the mean value of the ultimate plots. The equation

$$\sigma_w^2 = \sigma_p^2(1-r)$$

gives an identical value.

The coefficients of variation within combination plots are given in column 6 of tables 7 and 8. Table 7 gives the variability within plots for varying sizes when certain definite shapes are considered. Variability within combination plots decreases with a decrease in size when the shape remains the same. This result might be expected because contiguous plots tend to yield alike. As the intraclass correlation coefficient increases in value, the variability decreases within the combination plot. This follows from the relationships in the equation

$$\sigma_w^2 = \sigma_p^2(1-r).$$

Table 8 shows the data arranged with size as the constant factor but with shape allowed to vary. Here the intraclass correlation increases and the variability within combination plots decreases as the shape of the latter approaches a square.

The data show that the size and shape of combination plots have an important bearing on the intraclass correlation and variation

within them. Hence, when studying the size of combination plots in relation to the intraclass correlation and the variation within them, one should keep the shape constant. Conversely, when shape is being studied, the size should be held constant.

TABLE 9.—*Analysis of variance of grain yield between and within combination plots compounded from 16 ultimate plots and having a width : length ratio of 8/30=0.2667*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	Coefficient of variation
Between combination plots	89	8, 393, 855	94, 313 0	307 10	12.99
Within combination plots	1, 350	6, 248, 202	4, 628. 3	68. 03	11. 57
Total	1, 439	14, 612, 057	10, 175 2	100 87	17 16

NONCONTIGUOUS DISTRIBUTION OF REPLICATES

The only difference in studying intraclass correlation and variation within combination plots under this system and under the one just considered is in the distribution of the replicates. In the former study, where plot size and shape were emphasized, the replicates were distributed by contiguous association. In the present case, the replicates are distributed by noncontiguous association. The study has been carried out with two types of distribution: The replicates are distributed (1) completely at random and (2) at random within certain arbitrary limits. For 24 replicates, as an example, under the latter system of distribution, each of the 12 series was divided so that rows 1 to 60 constituted one group and rows 61 to 120 another. This gave 24 groups in all. One replicate then fell at random within each of these groups. Under the first system of distribution the replicates fell completely at random within the 1,440 rows used.

The data secured from this study appear in table 10. Under both systems of distributing replicates, the variation within combination plots of all sizes is always higher than 17.16, the coefficient of variation for the 1,440 ultimate plots in the study. This result was surprising, as the coefficients had been expected to vary around 17.16 as a mean. The explanation is that in every case a small negative intraclass correlation exists, which, when considered, enables one to calculate a theoretical figure agreeing with the one actually found. When the replicates are distributed completely at random, none of the intraclass coefficients, though all are negative in sign, is significantly different from zero. When, however, the replicates are distributed at random within arbitrary limits, all the intraclass correlations are again negative in sign and significantly so. The significance of the intraclass correlations was tested by the z test of Fisher (5).

For replicates distributed at random within arbitrary limits, the trend is definitely toward reducing both the coefficient of variation and the intraclass correlation coefficient as the number of replicates is increased. Perhaps no such trend exists when the replicates are distributed completely at random.

TABLE 10.—Actual and theoretical coefficients of variation of grain yield within and between combination plots, and intraclass correlation coefficients, when the replicates are distributed (1) completely at random and (2) within certain arbitrary limits

REPLICATES DISTRIBUTED COMPLETELY AT RANDOM

Replicates (number) ¹	Coefficient of variation within combination plots			Coefficient of variation between combination plots			Intra-class correlation (r)	Is r significant? (z test)
	Actual	Theoretical when $r \neq 0$	Theoretical when $r = 0$	Actual	Theoretical when $r \neq 0$	Theoretical when $r = 0$		
1.....				17.16±0.33				
3.....	17.39±0.33	17.39±0.33	17.16±0.33	9.63±.31	9.90±0.31	9.90±0.32	-0.0275	No.
6.....	17.24±.33	17.24±.33	17.16±.33	6.82±.31	6.82±.31	7.00±.32	-.0104	No.
12.....	17.24±.33	17.24±.33	17.16±.33	4.67±.30	4.67±.30	4.95±.32	-.0099	No.
24.....	17.19±.33	17.19±.33	17.16±.33	3.35±.31	3.35±.32	3.50±.32	-.0036	No.
48.....	17.21±.33	17.21±.33	17.16±.33	2.06±.27	2.06±.27	2.48±.32	-.0066	No.
60.....	17.22±.33	17.22±.33	17.16±.33	1.70±.25	1.70±.25	2.21±.32	-.0070	No.
180.....	17.18±.33	17.18±.33	17.16±.33	.89±.22	.89±.22	1.28±.32	-.0029	(²)

REPLICATES DISTRIBUTED WITHIN CERTAIN ARBITRARY LIMITS

1.....				17.16±0.33				
2.....	18.71±0.36	18.71±0.36	17.16±0.33	10.92±.29	10.92±0.29	12.13±0.32	-0.1898	Yes
3.....	18.03±.35	18.03±.35	17.16±.33	8.80±.29	8.80±.29	9.90±.32	-.1052	Yes.
4.....	17.76±.34	17.76±.34	17.16±.33	7.60±.28	7.60±.28	8.58±.32	-.0716	Yes.
6.....	17.66±.34	17.66±.33	17.16±.33	5.88±.27	5.88±.27	7.00±.32	-.0592	Yes.
12.....	17.47±.34	17.47±.34	17.16±.33	3.81±.25	3.81±.25	4.95±.32	-.0370	Yes.
60.....	17.26±.33	17.26±.33	17.16±.33	1.15±.17	1.15±.17	2.21±.32	-.0124	Yes.
180.....	17.18±.33	17.18±.33	17.16±.33	.87±.22	.87±.22	1.28±.32	-.0030	(³)

¹ Number of replicates refers to the actual number of ultimate plots compounding a combination plot.

² Standard error.

³ The number of combination plots is too small for an accurate test.

The existence of negative intraclass correlation coefficients has more than a passing interest. The negative correlation results from a reduction of the product moment in the equation for the calculation of r . That a negative r exists for combination plots compounded from two ultimate plots selected at random within arbitrary limits is shown by the contour map (fig. 2). Here the field was divided so that series 1 to 6 constituted one group and series 7 to 12 the other. The map shows that as combination plots are made by selecting at random one plot from each group, a high-yielding plot of the first group may often be combined with a relatively low-yielding plot in the second. When such pairs are entered in a correlation table in excess of the normal amount as expected from a normal distribution solely on the basis of chance, then a negative value is obtained. Perhaps when the means of the two groups differ significantly, a negative intraclass correlation coefficient follows. In the particular case considered, the means did differ significantly, and the value of -0.1898 for r (table 10) is significantly different from zero.

VARIATION BETWEEN COMBINATION PLOTS COMPOUNDED BY DIFFERENT REPLICATION SYSTEMS

Sometimes larger units than the ultimate one used in these experiments are desired. To investigate this phase of the problem the coefficients of variation for the larger plots were calculated. As in the preceding section, it is convenient to treat the variation between combination plots when they are formed (1) from contiguous ultimate plots and (2) from noncontiguous ultimate plots. In the

former instance size and shape may conveniently be discussed in relation to variation, and in the latter the number and the systems of distribution of ultimate plots, as replicates in relation to variation.

CONTIGUOUS DISTRIBUTION OF REPLICATES

It is to the variability between combination plots that most investigators have devoted their attention. In order to study this phase of the problem, the coefficients of variation were calculated for different sizes and shapes of combination plots. The data for the relation of size to variability, with shape constant, are given in table 7, and for the relation of shape to variability, with size constant, in table 8. The data show that the variability diminished as the size of plot was increased for any particular shape, and also as the shape of plot became long and narrow for any particular size. The results

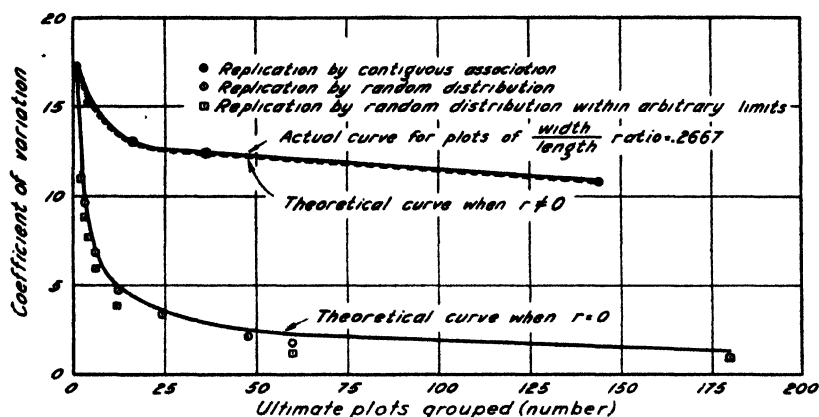


FIGURE 7.—Coefficient of variation between combination plots in relation to replication by contiguous and noncontiguous association for various numbers of ultimate plots grouped. It is possible to calculate two additional theoretical curves, one of which will pass through the circles and the other through the squares, representing the reduction in variation under two different systems of noncontiguous replication for various numbers of ultimate plots grouped.

emphasize the importance of keeping shape constant when studying size and size constant when studying shape.

The upper line (solid) of figure 7 shows the relationship between size of combination plot and variation for a particular plot shape of the width: length ratio = 0.2667. For simplicity's sake the curves for the other plot shapes are not shown. They are, however, similar in type. The lower line (solid) (fig. 7) is the theoretical curve expected on the basis that the reduction in variation is proportional to the square root of n , the number of ultimate plots grouped. A wide discrepancy appears between the two curves. However, by allowing for the correlation that exists among the ultimate plots compounding the combination plots, a second theoretical curve can be constructed which agrees perfectly with the actual results obtained. This curve is the dotted line just below the solid line in figure 7. Actually the two curves are identical, but on the planar graph they had to be drawn parallel to each other. The reduction in variation is therefore a function of n , the number of ultimate plots grouped, and of r , their intraclass correlation coefficient. The equation for computing the variation as a function of n and r has already been cited.

NONCONTIGUOUS DISTRIBUTION OF REPLICATES

The usual practice in agronomic field experiments is to distribute several replicates of the treatments or varieties over the area on which the tests are made. In studying this type of procedure, combination plots were compounded with different numbers of replicates or ultimate plots distributed in different ways. Of the many possible systems of distributing the replicates only two are investigated in this study: (1) That in which distribution is completely at random and (2) that in which distribution is at random within certain arbitrary limits. Replicates may fall next to each other by chance, but such cases are exceedingly few and will not influence the results to any marked degree.

The results of this study appear in table 10. The data show that the reduction in variation, as the number of replicates is increased, is much greater under this system than where the replicates are distributed by contiguous association. This fact is shown graphically in figure 7. The reduction in variation is nearly proportional to the square root of the number of replicates used. In every case investigated, the actual reduction of variation was slightly greater than that expected on the basis of a zero correlation coefficient between the replicates of the combination plots. These discrepancies are caused by the existence of small negative intraclass correlation coefficients, the values for which are given in table 10. When these coefficients are considered, the theoretical figure for the variation agrees exactly with the one actually obtained. Table 10 shows this in columns 5 and 6. Two theoretical curves can therefore be calculated, one passing through the circles and the other through the squares of figure 7.

When the same number of replicates was investigated under both systems of distribution mentioned above, the reduction in variation was always slightly greater where the replicates were distributed at random within certain arbitrary limits. The difference is explained by the value of the intraclass correlation coefficient, the only factor left to vary when two systems involving the same number of replicates are compared. If the equation for computing the intraclass correlation coefficient is examined, the only variable that changes, r , evidently is the product moment part, as all the other variables remain constant. Hence, if one can explain why the product moment changes for different systems of distribution, the difference in variation follows logically. For the sake of brevity, it will suffice to show how the product moments differ for the 2 systems when only 2 replicates are considered. Suppose the 2 replicates compounding a combination plot yielded alike, say 500 g each. Then the product (500×500) will be a maximum. Any other division of the yields of the replicates, say 490 and 510, will give a product of lower numerical value. This unequal division of the replicates compounding a combination plot (an inequality too great to be caused by chance alone in drawing at random from a normally distributed population) probably explains the difference in the product moments. To illustrate more specifically, let the first replicate be selected from series 1 to 6, the second from series 7 to 12, both selections being at random within these areas. Now the mean yield of the ultimate plots of series 1 to 6 is 625.22 ± 3.77 g; of series 7 to 12, 550.55 ± 2.96 g. The difference (74.67 ± 4.79 g) is highly significant. The product moment formed by selecting the

first replicate from a group ranging around 625.22 as a mean and the second from one ranging around 550.55 is less than the product moment from two replicates selected at random around the grand mean of 587.89 ± 2.58 g. Obviously, the average difference between the means of the first and second replicates, each selected completely at random, would not exceed some 15 times its standard error except in a very large number of trials.

As shown earlier in this paper, adjacent rows are positively correlated, and significant positive correlations are found between rows up to a distance of 48 feet apart. Now the establishment of arbitrary limits on a field of this kind tends to make the variation of the means (derived from the ultimate plots within the limits) greater than if the same number of ultimate plots were selected at random. Since the means vary more, the product moment of the intraclass correlation coefficient of single replicates selected at random from within these delimited groups will be less, for the reasons just stated. Hence the variation between combination plots compounded by a systematic distribution of the replicates will usually be less than where the replicates are distributed completely at random.

THE PRINCIPLE OF MAXIMUM CONTIGUITY AND ITS PRACTICAL APPLICATION

According to Student (1, pp. 274-275), the principle of maximum contiguity in field-plot technic was deduced a priori by E. S. Beaven. Although the principle is not formally defined, its essence is contained in the following statement by Student:

* * * naturally the nearer two plots are together the more likely is the soil and its condition to be similar on each of them, and the obvious conclusion may be drawn that the smaller the plots the more exactly can the yield of adjacent plots be compared.

Applied to the data under consideration, the principle may be stated as follows: When the yields of nearby plots are correlated, the mean distance between the contiguous ultimate plots compounding combination plots has an influence on the variability within and between the latter. Hence the nearer the ultimate plots are together in a combination plot of a given size, the less will be the variability between these plots.

The question as to the best size and shape of plot on which to place a single replication of a number of varieties may be approached on the principle of maximum contiguity, assuming that the variance between replications can be eliminated by methods given by Student (1), Fisher (5), or Engledow and Yule (4). Suppose three varieties of wheat are to be tested for their yielding ability. In the United States, the ultimate unit of small-scale testing is usually a three-row block, 15 to 18 feet in length, of which only the center row is harvested. The rows are usually 12 inches apart, and sometimes their ends are discarded before cutting to eliminate any border effect. A total of nine rows then will be needed to test the three varieties, which are distributed at random in each replication. In table 8 the nearest size of plot investigated contains eight rows. Using this size in ascertaining the best arrangement in which to grow the rows, one finds that the coefficient of variation of the rows within the plot for this size decreases in proceeding from a long narrow plot (width: length ratio 0.0333) to one more nearly square (width: length ratio 0.5333). The actual coefficients are 11.93, 11.32, and 9.70. Obvi-

ously, the plot with the smallest coefficient (9.70) would be best for testing the three varieties.

As another example, suppose 24 varieties are to be tested. This will require a plot of 72 rows. Three shapes have been investigated for this size, with results shown in table 8. Here again, the more nearly square the plot the smaller will be the coefficient of variation. Under the restriction that all the land had to be used whenever a particular grouping was studied, a plot shape more nearly square could not be made for a size of 72 rows. If the general trend of variability is at all indicative, one would expect a lower figure even than 12.40 (this is for a width: length ratio of 0.5333) for a square plot.

The following general procedure seems to be a practical application of the principle of maximum contiguity when the yields of numerous varieties are to be tested. As previously shown in this study, the variation of ultimate plots within a combination plot compounded from contiguous replicates of a given number decreases as the combination plot changes in shape from long and narrow to square. Within square combination plots, the variation of the ultimate plots decreases as their number decreases. One must therefore select the number of varieties that are to be grouped into one replication of the test, and then arrange them to form a combination plot as nearly square as is practicable. In the present case, five varieties would seem a desirable number to select. One replication of these would occupy 15 ultimate plots, and they could, by the principle of maximum contiguity, be arranged into a square combination plot. In order not to introduce a bias in estimating the experimental error, the varieties in any one replication will be assumed to be arranged at random. All the varieties, in groups of five, can then be investigated as to yielding ability. It seems also advisable to include in each group of five a standard or check variety which is the same for each group. The experimental error is perhaps best estimated by an analysis of variance, deducting the variance due to replications and varieties from the total variance in arriving at the experimental error. If a very large number of varieties are to be tested, the experimental error need not be calculated for each group of 5; but the average error from several groups of 5 may be applied throughout all the groups of 5 varieties. The only valid use of the error is, of course, in comparing the yields of the five varieties in a group. Of particular interest is the comparison of the standard or check variety with each of the four other varieties in the group.

In another plan that seems to have some points of merit, the varieties are planted in sequence 1, 2, 3, . . . n , and repeated in like manner for all subsequent replications. Every fifth, tenth, fifteenth, twentieth variety, etc., is a standard variety or check. The experimental error is estimated in the same manner as in the preceding case. The present arrangement permits the error to be applied as a "moving error" for any contiguous group of five varieties. For instance, the average yields of varieties 6, 7, 8, 9, 10 may be compared with each other. The same is true of varieties 7, 8, 9, 10, 11, 12; 9, 10, 11, 12, 13, etc. The "moving error", which has the same percentage value throughout the experiment, permits each of the four varieties between the checks to be compared with both those checks. For example, variety 8 can be compared with the check varieties at 5 and 10. Often two check varieties are used alter-

nately, as when the hybrids of a cross are compared with the parents. The type of planting suggested here is well suited for that purpose. The efficacy of this method to reduce the experimental error depends on the value of the intraclass correlation coefficient. A valid objection to this planting scheme is that it is systematic and may therefore introduce some bias into the experimental error. A slight bias in the error might, however, be easily outweighed by other practical considerations, such as ease in planting, harvesting, etc. Further studies on this point are needed.

DISCUSSION

When the general variability in grain yield within a field is investigated by adding more plots of the same size and shape to enlarge the field, Stringfield (20) and Stadler (18) showed that the total variation has a tendency to increase. In this paper such a procedure is called the extended system of sampling. Stringfield studied the variation of increasing field sizes when the plot sizes varied from those customary in nursery work to 0.1 acre. In all cases, he found the total variation increased as more and more land was brought into the experimental area. Both wheat and oats were used in his studies. Stadler, working with nursery plots of the same grains, had similar results. The data of the present study agree with those of Stringfield and Stadler. Since the variation and mean yield of the plots to be added to the experimental area are unknown, the variation of the enlarged area cannot be predicted. Apparently the only conclusion warranted by the data at hand and by those of others (18, 20) is that the total variation will perhaps be greater for the larger area than for the smaller one. Change in variation of increased areas depends on the relation of the mean and variation of the additional plots to those already in the experiment.

Under another type of extended system, where more and more land is brought into the experimental area by increasing the size (in this study, lengthening the individual rows) of the original plots, the total variation tends to decrease. Stringfield (20) studied a comparable case with similar results. Here, again, the actual variation cannot be predicted beforehand, because the character of the land added is not known. In part, at least, the reduction occurs because positive and negative deviations cancel each other as adjacent plots are added together and because the variability of a series of averages (adjacent plots added together) tends to be less than that of the single plots.

When the variability of different row lengths is investigated and all the land is used for each study (the closed system), the variability decreases as the rows become longer. From a practical viewpoint of field-plot technic the question might be asked, what is the best length of row to use? The length of row in nursery practice has usually been determined by convenience: 16 feet for wheat, 15 for oats, and 20 for barley. The yields from these row lengths, taken in grams, are easily converted into bushels per acre. It seems rather anomalous that the row lengths in common practice should have been selected on such a criterion. As shown by figure 5, the curve for the coefficient of variation is almost a straight line, although the reduction in going from a 15-foot to a 30-foot row is a little greater than for any other part of the curve. The present study does not seem to justify a departure from the row lengths now used.

The correlation between adjacent rows and those at greater distances has been investigated by Hayes (12) and Griffiee (8). Hayes, studying wheat and oats, computed the correlation for rows separated by 3, 6, 9, 12, 15, and 30 feet, respectively. He found that the correlation decreased as the distance between rows was increased. Griffiee, working with wheat, calculated the values of r for rows up to 10 feet apart. Though this is only a short distance, still the values he found seemed to be smaller as the distance was increased. On the whole, the values obtained in the present study agree with those of Hayes and Griffiee. They are higher for comparable distances between rows, but this difference may not be significant, as there is not a priori reason why they should be equal. A possible influencing factor may be that Hayes and Griffiee correlated row lengths slightly longer than those in this study; they used rod rows, whereas in this study the row lengths were 15 feet. According to the data at hand, the value of r diminished as the distance between plots increased; and the resulting curve, shown in figure 6, is very nearly a straight line. Significant correlations were not demonstrated beyond 48 feet.

After Harris (10, 11) developed a short-cut method for calculating the intraclass correlation between the ultimate units compounding a combination plot, this feature of field-plot technic could be studied. The method is applicable to combination plots where the replicates are distributed either contiguously or noncontiguously. The former distribution has been used in the most extensive studies made. Harris has devoted a good deal of attention to this matter. He computed the coefficients from a relatively large number of uniformity trials distributed throughout the world and planted to a considerable variety of crops. The coefficients ranged from +0.020 to +0.830 for adjacent plots. Harris was able to show that the correlation decreased as the number of ultimate plots in a combination plot increased. Garber, McIlvaine, and Hoover (7), working with wheat, also found the correlation to decrease as the number of ultimate plots in a combination plot increased. The data from the present study agree with those just mentioned (7, 10, 11). For the particular crop studied (wheat) they have been extended to cover a greater range of combination plot size and shape. Heretofore, plot shape has been largely disregarded when the intraclass correlation for varying sizes was being studied. These experiments emphasize the fact that plot shape is an important factor in determining the numerical value of the coefficient. For any given size of combination plot the intraclass correlation coefficient became greater as the shape approached a square. The data showing these relationships are given in tables 8 and 9.

The variation of the ultimate plots within a combination plot has a simple relation to the intraclass correlation coefficient, as is shown by Fisher (5). The relation is an inverse one; that is, as the correlation of the ultimate plots of a combination plot increases, their variation decreases. The variation, therefore, decreases when the shape is constant but the size decreases, and similarly when the size is constant but the shape approaches a square (tables 7 and 8).

The intraclass correlation coefficient and the variation within combination plots were also calculated when the replicates were distributed noncontiguously. Garber, McIlvaine, and Hoover (7), investigating some wheat-yield data in this way, concluded that the intraclass correlation for replicates thus distributed was sensibly zero.

The distribution in their case was systematic. In the present study, two types of distribution were used: The replicates were distributed (1) completely at random and (2) at random within certain arbitrary limits. The resultant data are given in table 10. The intraclass correlation coefficients for both types of distribution proved to be much smaller than when the replicates were contiguous. When distribution is completely at random the values for r are not significantly different from zero, but when it is systematic they are significantly different. The variation of the ultimate plots within combination plots is in these instances also inversely related to the intraclass correlation, as indicated in the preceding paragraph. Care must be taken, however, in interpreting the present cases, as the sign of r is negative.

The variation between combination plots has received most attention from investigators. It is discussed separately for combination plots formed by contiguous and noncontiguous distribution of the replicates. Mercer and Hall (15), working with wheat combination plots distributed contiguously, found variability decreasing as plot size increased. Other investigators who obtained similar results with wheat are Montgomery (16, 17), Forster and Vasey (6), Day (3), Summerby (21), Stringfield (20), and others. In the present study similar results were obtained; as the size of plot was increased for any particular shape, the variability decreased. The data are given in table 7. A general criticism that might be made of some earlier work is that the plot shape was not kept the same when size was investigated in relation to variability.

Lyon (14) was perhaps the first to point out that plot shape is an important factor in plot variability. Analyzing the data from the Mercer and Hall (15) wheat trial, he showed for the two plot shapes studied that long, narrow plots were more uniform than square ones. Day (3), studying the data from a wheat uniformity trial, reached the same general conclusions. He found the long, narrow plot of particular advantage if its greatest dimension extends in the direction of greatest soil variation. An objection to Day's analysis is that he kept his plot size only within certain limits when studying the effect of shape. Stringfield (20) concluded that among small-grain nursery plots increasing the length was distinctly more advantageous than increasing the width. The most extensive examination of plot shape as related to variation was made by Christidis (2), who critically analyzed the results of eight uniformity trials and found that in no case were square plots less variable than long, narrow ones. In certain cases, the latter shape could not be shown statistically to have any advantage, but he concluded that those cases were open to criticism because certain mechanical errors were not kept properly under control. Other investigators of wheat who have found a reduction in variability for the long, narrow plot are Summerby (21), Forster and Vasey (6), and Kiesselbach (13). The results of the present study (table 8) agree with those just reviewed; they show that plot variability is influenced by both size and shape, and that one must keep size constant when studying shape, and vice versa.

The variation between combination plots compounded by noncontiguous distribution of the replicates has been studied by Mercer and Hall (15) and many other investigators. The efficacy of this method in diminishing the variation has not been seriously challenged by any one, so far as the writer is aware. The data of this study (table 10)

confirm the results of other workers. The reduction in variation is nearly proportional to the square root of the number of replicates used.

Tedin (22) has investigated eight uniformity trials from different parts of the world by different systems of distributing the replicates. He found that systematic arrangements introduce a bias in the estimated error of the experiment, and in general give estimates either over or under those derived from random arrangements. The data secured in this study also agree with his findings. Figure 7 shows that the variation for noncontiguous replicates distributed at random within certain arbitrary limits is always less than where distribution is completely at random. Practical considerations may, however, be more important than the slight bias introduced. More studies are needed on this point.

Many contributors to the subject of field-plot technic have had difficulty in harmonizing their actual curves with those based on the equation where the reduction in variation between combination plots is proportional to the square root of n , the number of ultimate plots grouped. Student (1), Engledow and Yule (4), and Stadler (19) have pointed out that the reduction in variation is proportional to the square root of n only if the intraclass correlation is zero. Where the combination plots are compounded from contiguously distributed replicates, the intraclass correlation usually has a significant positive value and hence the variation is reduced less than expected. On the other hand, when the replicates are distributed in a systematic non-contiguous manner, the correlation is more often negative in value and the variation is reduced more than expected. The present study shows that the actual and theoretical curves agree absolutely if the latter are calculated as a function of n and r . The equation expressing the relationship is given in the text. The relation of actual and theoretical curves is shown graphically in figures 5 and 7, and the data are given in tables 5 and 10.

Like the variation between combination plots, the theoretical variation within combination plots can be computed. A different equation, however, must be employed.

SUMMARY AND CONCLUSIONS

The total variation tends to increase as more land is added to the experimental area, provided the size and shape of the ultimate unit remain the same.

The total variation tends to decrease as more land is added to the experimental area and the size of the ultimate plot increases, provided the number of plots remains the same. In this connection the size of the plot was increased by lengthening the row.

When the entire experimental area is used in each study of the relation of variation to increase in row length, the variation tends to decrease and is a function of n , the number of ultimate plots combined, and r , their intraclass correlation coefficient. The change of variation under these conditions is nearly linear.

As the distance between the rows (the ultimate plots) increases, their correlation decreases. Statistically significant correlations could not be shown beyond 48 feet.

The intraclass correlation coefficient, calculated according to a formula of Harris, increases as the size of the combination plot (compounded by contiguous association) decreases, provided the shape remains constant. When the size of plot remains the same, the coefficient increases as the shape of the plot approaches a square.

The variation of the ultimate plots (rows) within a combination plot compounded by contiguous association may be secured from the intraclass correlation coefficient with the aid of an equation or from an analysis of variance. The two methods of approach lead to the same result.

The variation within combination plots compounded by contiguous association decreases when the shape is constant but the size decreases and also when the size is constant but the shape approaches a square.

The variation within combination plots compounded by noncontiguous association approaches the total variation. The nearness of approach depends on the value of the intraclass correlation. In this study the variation within the combination plots was slightly greater than the total variation because of the small negative values for r .

The variation between combination plots compounded by contiguous association increases when their shape is constant but their size decreases and also when their size is constant but their shape approaches a square.

The variation between combination plots compounded by noncontiguous association decreases as the number of ultimate plots grouped becomes larger. The reduction in variation is nearly proportional to the square root of n , the number of plots grouped.

The decrease in variation between combination plots is greater when the replicates are distributed over the experimental area than when they are distributed by contiguous association.

The discrepancy frequently found by agronomists between actual and theoretical curves for the decrease in variation between combination plots, as more ultimate plots are used, is shown to result from the correlation between the ultimate plots compounding the combination plots. The variation is a function of n , the number of ultimate plots grouped, and r , their intraclass correlation. When n and r are both considered and the correct theoretical curve is calculated with these as the independent variables, the actual and theoretical curves agree exactly.

When the replicates were distributed in a noncontiguous manner, complete random distributions gave less bias to the estimate of the experimental error than did systematic distributions.

The data indicate that a smaller experimental error is obtained by grouping the varieties of each replication so as to make the distance separating them a minimum. The application of this method, called the principle of maximum contiguity, permits a greater part of the total variance to be apportioned to that designated as "between combination plots", which can be eliminated statistically, and thus leaves a smaller portion for calculating the experimental error.

The data suggest the use of two systems of plot arrangement for nursery practices when the number of varieties to be tested is large. These systems involve the principle of maximum contiguity. The varieties to be tested are divided into groups of 5. One of the 5 in each

group is a standard variety, or check, and the groups are replicated the desired number of times. In one system, the varieties are distributed at random in each group; in the other, they are arranged in the same sequence in the replications. An experimental error is calculated by analysis of variance for each group of 5 varieties when the number of varieties tested is not too large. When many varieties are tested, the average error from several groups of 5 may be applied throughout all the groups of 5. When the varieties are planted in sequence, the experimental error may be used as a "moving error" and applied to any contiguous group of 5 varieties. The moving error permits any variety to be compared with the check, or standard variety, on either side.

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MORPHOLOGY AND BIOLOGY OF THE WHEAT JOINTWORM GALL¹

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INTRODUCTION

The study of the morphology of wheat-stem galls caused by the wheat jointworm, *Harmolita tritici* (Fitch), was begun by the senior writer in 1927 at Charlottesville, Va., but it was incidental to the investigation of the insect itself and the measures for its control. During the winter of 1932-33, however, it appeared that with more material available facts of definite economic importance might be revealed. In the spring of 1933, therefore, a large number of galls, collected during the period of development of the insect from hatching to maturity of the larva, were preserved at Charlottesville. During the previous winter, arrangements had been made with the laboratories located at Wichita, Kans., and Lafayette, Ind., to preserve wheat stems in their various stages of growth. Since then a comparative study of the plants from the three localities relative to their susceptibility to jointworm attack, has been made.

As late as 1904 F. M. Webster stated that *Harmolita tritici* was not known to occur west of the Mississippi River. Since that time it has been found in the Wheat Belt as far west as central Missouri and also in Utah, California, and Oregon. The results of this investigation may explain why the wheat jointworm does not occur in Kansas and how its ravages may be materially reduced in the Eastern and Central States.

HISTORICAL RÉSUMÉ

A large amount of work, mostly in Europe, has been done on the morphology and biology of insect galls. Anyone interested in this subject should consult the bibliographies given by Kinsey (6)³ and by Ross (9). Neither bibliography is complete, but each supplements the other. Ross does not list North American works. His book is very recent, however, and is of more general interest inasmuch as it covers the whole field of gall-forming organisms.

In reviewing the literature on gall-forming insects, one is struck by the small amount of work that has been done on the chalcids. This may be due to the fact that, so far as known, all chalcid galls are small and inconspicuous, and perhaps are found mostly on the grasses.

Only three published articles on the morphology and biology of *Harmolita* galls have been found. One paper (8) deals with galls on *Ficus* spp. and *Cattleya* sp.⁴ and the other two (3, 5) are on the terminal and axillary bud galls of *Agropyron* spp. These papers are of little

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³ Reference is made by number (italic) to Literature Cited, p. 385.

⁴ These gall makers, in all probability, do not belong to the genus *Harmolita*.

interest in the present study, since they involve different species and different parts of the plants, and the plant responses are very different. Besides, the wild grasses involved continue growing throughout the season, as is largely true of the bud galls on them, whereas the wheat jointworm galls mature slightly in advance of the wheat plant.

METHODS AND PROCEDURE

In order to make a complete study of the morphology and biology of the galls produced by *Harmolita tritici*, tissues were preserved daily from the moment the eggs were deposited until the larvae became full grown. To accomplish this, wheat plants were transplanted to 12-inch flowerpots, and each plant was labeled and enclosed within a 10-inch glass cylinder, the upper end of which was covered with cheesecloth (fig. 1, *A*). From 30 to 40 females were then introduced into each cage, and the place of oviposition on the culms and the approximate number of eggs deposited were recorded. No female was allowed to remain in a cage longer than 8 hours, frequently half that period being sufficient for infestation of all susceptible culms. Plants in the field also were covered with cylinders in the same manner, and oviposition was recorded. These field plants were used principally for studying the later instars of the larvae and the older gall tissue.

Fixing solutions of several kinds were used, but the formalin-acetic-alcohol mixture gave the best results. The tissues were demineralized in 10-percent hydrofluoric acid for 3 days, dehydrated, and cleared according to Zirkle's (18) butyl-alcohol method. Hance's (4) paraffin-rubber mixture was used for embedding during the winter of 1933-34. In previous work the old method with xylol and ordinary paraffin had been followed, but the butyl alcohol and paraffin-rubber combination was found to be superior. Sections were cut to a thickness of 14μ and stained in iron-alum haematoxylin. Gentian violet, acid fuchsin, and orange G were used as counter stains. The violet was found to be a more satisfactory counter stain for the older tissues.

OVIPOSITION

DEPOSITION OF THE EGG

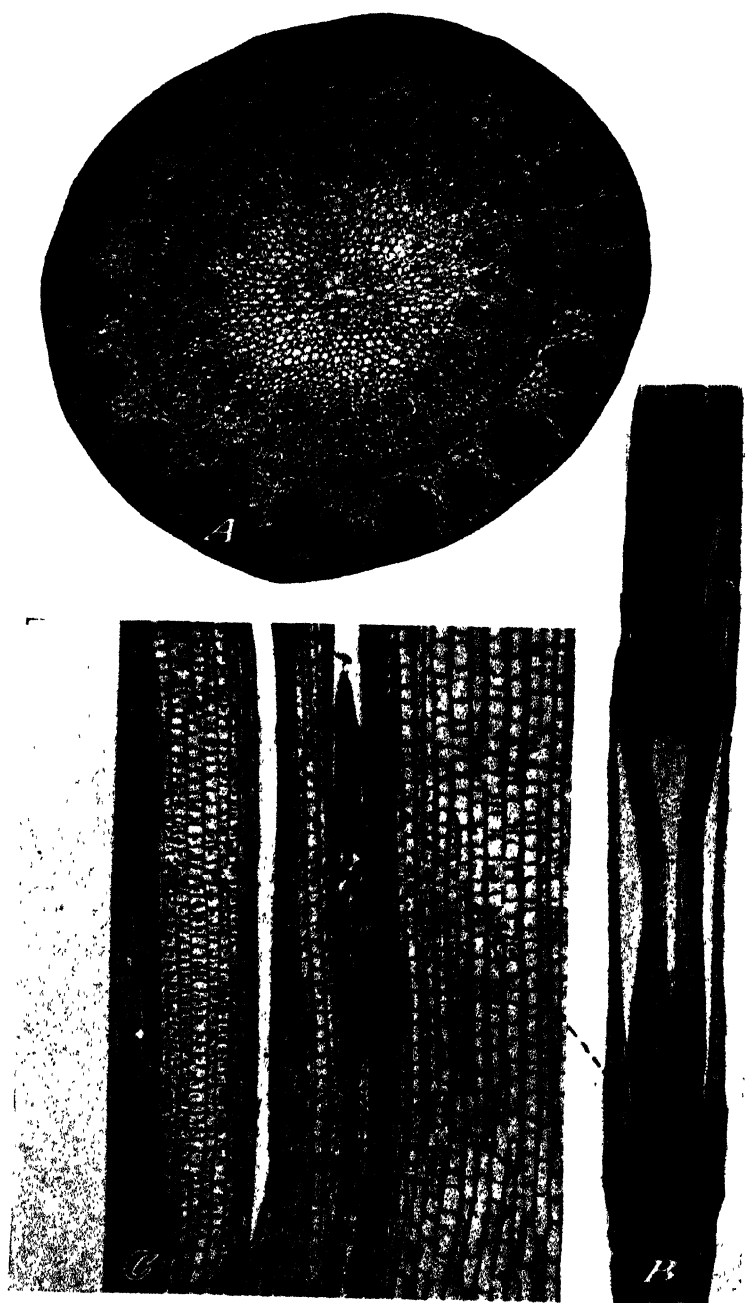
Harmolita tritici oviposits in the rapidly growing culms of wheat after these have reached a certain critical stage of development. When the complete mechanism of oviposition is thoroughly understood it becomes at once one of the most interesting and, perhaps, most important phases of the whole subject of wheat jointworm gall formation. It will, therefore, be treated at some length.

The female locates a suitable point for oviposition by walking slowly up and down the wheat culm, tapping its surface continually with her antennae as she moves along. This process may occupy considerable time even when a culm in the proper stage of growth is under examination. The place selected for oviposition is at the upper side of a node, in tissue that will later form the internode. The ovipositor (fig. 1, *B*) is inserted slightly below the meristematic region, at the union of a leaf base with the stem (fig. 2, *B* and *C*).

It will be noted from figure 1, *C* and *D*, that there is as yet no visible evidence of the node at the point of oviposition, as the internode below has not lengthened sufficiently to elevate it into view.



FIGURE 1.—A, Breeding cage; B, the spearhead of the ovipositor lance, $\times 628$; C, female *Harmolita tritici* in the first position for oviposition, $\times 2$; D, female in the final position and in the act of oviposition, $\times 2$; E, internode that was too old and tough at the time of oviposition to respond properly to the stimuli incident to the presence of the larvae, and the expanding gall tissue ruptured the epidermis, expelling a larva (p. 367), $\times 6$; F, different types of galls that may be found in any wheat field infested with *H. tritici*, the long gall at the left is the type most frequently found, and the two warty galls at the right are the kind that develop from the type illustrated at E in which the larvae rarely mature; almost natural size.

**FIGURE 2**

(For explanatory legend see opposite page)

That is, the node at the oviposition point is covered by the sheath of the next lower leaf. The only way one can definitely locate such a node without removing the sheath is by pressing the stem between the fingers. Occasionally females do attempt oviposition at an exposed node where the tissue is older and harder, but such nodes are difficult to pierce, and larvae do not mature in this older tissue.

After selecting the point for oviposition, the female, with her head directed down the stem, lowers the abdomen to the surface of the culm, and, at the same time, she brings the tip of it forward until the abdomen is at right angles to the thorax (fig. 1, *C*). The ovipositor is pointed into the tissues and the abdomen is elevated to almost a vertical position. The ovipositor is used very much as a ginlet to bore a hole through the leaf sheath (fig. 2, *A*) into the tender tissues of the culm, slightly below the meristem of the internode, until a vascular bundle is reached within the culm. By referring to figure 3, *C*, it will be seen that the phloem lies beside and external to the xylem in a vascular bundle of the collateral type. The ovipositor passes through the phloem until it reaches the xylem tubes, the hardest elements of the internal tissues at this point, then it turns upward, following the tubes (fig. 2, *B*) until the hole made by the ovipositor is of sufficient depth. At this time the insect is in the position shown in figure 1, *D*. The female remains in this position for several minutes in order to allow the egg to pass down the oviduct and into the tissues of the plant. The ovipositor is then partially withdrawn and thrust back into the tissues in a different direction (fig. 2, *A*) in search of another vascular bundle, where an additional egg is deposited. This latter process may be repeated 5 or 6 times, resulting in the deposition of as many eggs through one external opening. It may be seen (fig. 2, *A*) that all the eggs cannot be placed in the same horizontal plane, even though the same external opening is used. Those eggs placed in the vascular bundles immediately under the body of the female will be highest in the internode as they are placed at the farthest point attainable, and those deposited in the bundles to her right or left will be lower. The insect doubtless finds the epidermis difficult to pierce with the ovipositor and probably saves a great deal of energy by depositing several eggs through a single opening.

After oviposition is completed at the first external puncture, the female frequently moves around the stem in the same horizontal plane (fig. 2, *A*) and bores a second hole to insert more eggs. Occasionally as many as 16 eggs are deposited in the meristematic tissue immediately above a node (fig. 3, *A*). Figure 3, *B*, shows a highly magnified vascular bundle with an egg in place and almost against the xylem tube, and figure 3, *C*, shows a normal bundle of the same age. The average in nature, as shown by dissection of many mature galls, is about 6 or 7 larvae to the infested internode.

EXPLANATORY LEGEND FOR FIGURE 2

- 4, Cross section of a wheat culm, at the top of a node, through an oviposition puncture 1 day old. Note how the channel branches towards the vascular bundles; below this, and farther around the stem, may be seen traces of other channels emanating from another puncture that does not show in this section. On the opposite side of the stem may be seen still fainter traces of channels from another oviposition puncture that is not shown; $\times 25$. *B*, A longitudinal section of the three upper nodes and the embryonic head of a wheat culm. All the three nodes and internodes together measured about one-half inch. The dotted line indicates the position of the day-old egg in the meristem, just above the node; $\times 7.8$. *C*, The egg and the surrounding meristematic tissue shown in *B*, but more highly magnified; note how closely the oviposition channel follows the xylem tubes and that the egg lies in the phloem with one side almost against the xylem tubes; $\times 58$.

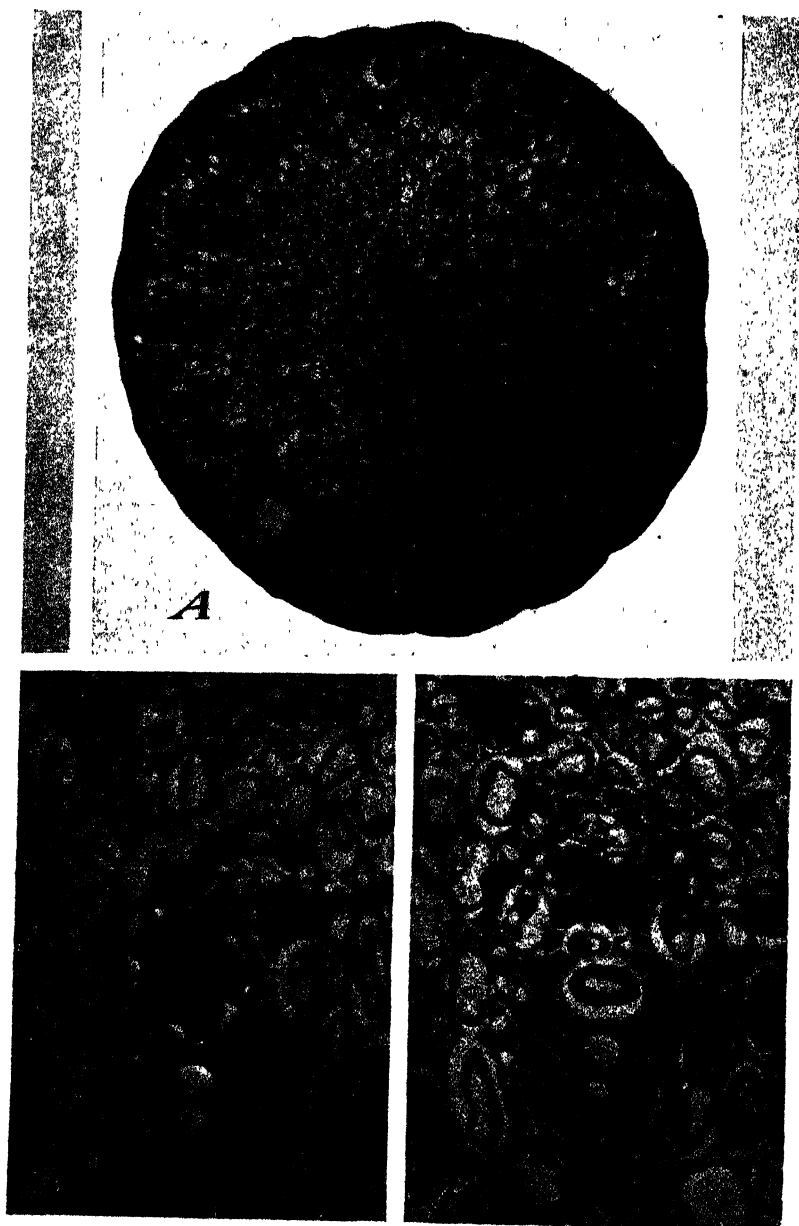


FIGURE 3.—*A*, Cross section of internode containing 16 day-old eggs, $\times 64$. All the eggs are in the phloem of the vascular bundle and there are only three large bundles that do not contain eggs. *B*, Vascular bundle 1 hour after oviposition, $\times 330$. *C*, Normal vascular bundle from the same section as *B*, $\times 330$.

The eggs are always found in the phloem of the vascular bundle (fig. 3, *A* and *B*), or in the parenchyma immediately adjoining it. Numerous galls have been sectioned, involving the cutting through of many eggs, and, with perhaps one exception, the female has never been found to miss the mark; and in this exceptional case the egg was located very close to the phloem. Although the eggs are usually placed in the large bundles, an egg may occasionally be found in one of the small vascular bundles near the epidermis; however, even here the egg is properly placed in the phloem.

The writers have never found a record in literature to indicate that any female gall-making insect selects the phloem for the placement of the egg. This, of course, is the most favorable location for the placement of the eggs, since the prospective larvae would be surrounded by rich food material immediately upon hatching. Other gall-making insects select various parts of the host plant, the eggs of some being placed even on the surface of leaves, the gall tissue growing up around them from all sides. A number place their eggs in the cambium layers and others under the growing points of buds. In general it appears that the vascular system of gall tissue is simply a modified extension of the regular vascular system of the neighboring normal tissue, whereas in the case of the wheat jointworm gall the normal vascular system of the internode involved, as later described, is almost entirely destroyed (figs. 4, *B*, *C*, and 11, *A*, *B*).

The fact that the eggs are precisely placed in the phloem makes oviposition a tedious operation. If the female could puncture the meristem at any point without regard to its age and texture and could insert the eggs in a haphazard manner, the process would be greatly simplified. It is necessary for her to probe the tissues carefully to locate the vascular bundle in which to place each egg. Since only one egg is placed in the phloem of any bundle, a fresh bundle must be sought for the deposition of each individual egg. The larvae thus find themselves so placed as to intercept the rich sap flowing in the main conduction channels carrying elaborated foods and, therefore, have the first call on the resources of the plant.

FACTORS INFLUENCING OVIPOSITION AND THEIR RELATIONSHIP TO CONTROL MEASURES

The most important factors influencing oviposition are temperature, the size of the host plant, and the structure of the meristematic regions of the culms where the eggs are to be placed.

Oviposition occurs very sparingly at temperatures below 70° F. and is most active at or a little above 80°. Regardless of temperature, adults are inactive at night and during stormy weather. They are rather frail creatures and heavy rain and wind storms are very disastrous to them. Under the most favorable circumstances a female may deposit the majority of her eggs and die at the end of 2 days. Apparently no eggs develop after the female emerges. Cold, cloudy weather may prolong the lives of the adults for an additional 3 or 4 days.

The portion of the culm usually most attractive for oviposition is only about one-fourth inch in length, or, if there should be two more nodes above this, all three together at this time would measure about a half inch (fig. 2, *B*), and it is rare that on any one culm there will

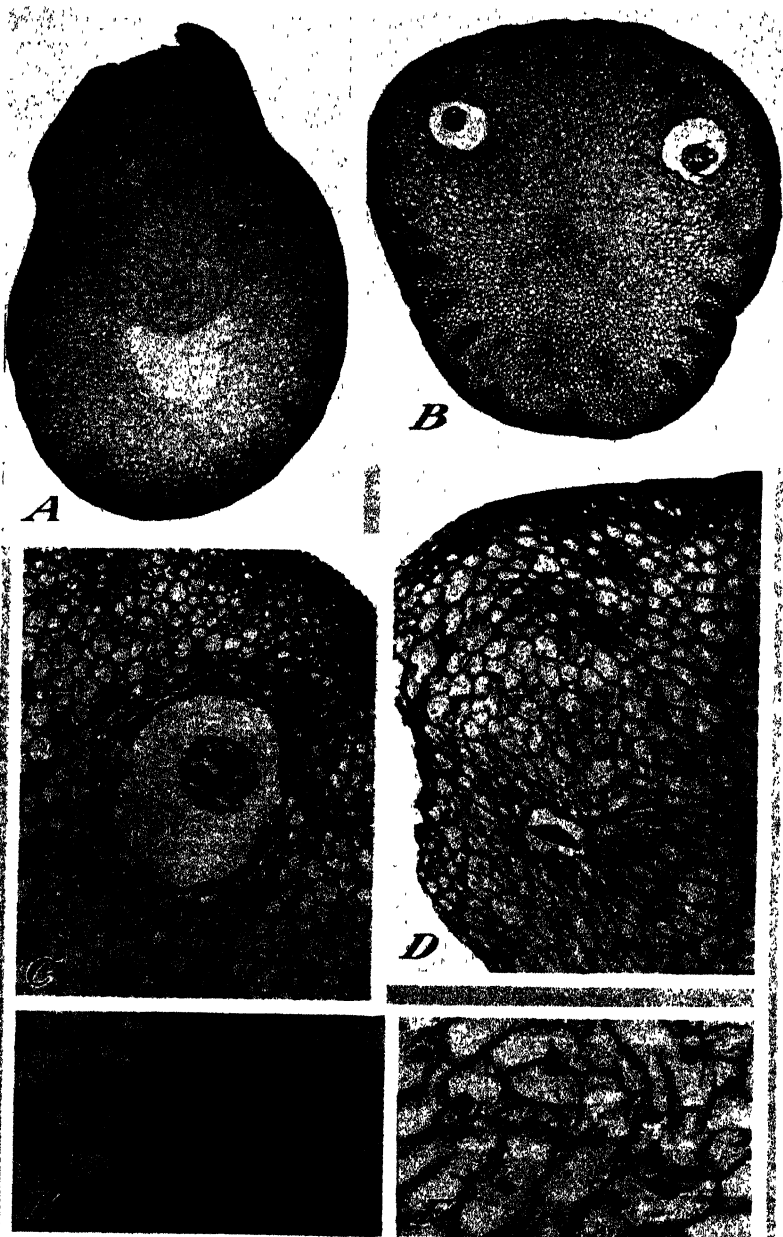


FIGURE 4.—*A* and *B* are transverse sections of two galls from the same culm during the second larval period. The eggs for each gall were deposited the same day, and the tissues were preserved in a fixing solution when at the same age. The gall from which *B* was cut was the internode immediately above *A*, and therefore the younger and more tender internode. Note that the larvae and the gall tissue have developed normally in *B*. The tissue in *A* was too old to respond to the stimulus incident to the presence of the larvae, and normal gall tissue did not develop; *A*, $\times 18.7$, *B*, $\times 23.6$. *C*, Normal food tissue surrounding normal larvae shown at the upper left in *B*, $\times 64$. *D*, A portion of *A* at higher magnification; all larvae are dead in this internode and no rich food tissue surrounds them; the larvae starved and died in consequence, $\times 44$. *E*, Normal parenchymatous tissue outside the gall area of the second larval instar, $\times 138$. *F*, Section illustrating loss of polarity in gall tissue of the second larval instar, $\times 150$.

be on one day more than one attractive node. Regardless of favorable weather conditions the females will ordinarily not oviposit in any but attractive material, but frequently a female will work industriously in an effort to oviposit in a second internode and may succeed in placing several eggs. Larvae hatching in such positions cause warty or irregular deformations, as shown in the two very short galls in figure 1, *F*. Rarely do such larvae reach maturity because the tissues in these locations are too old to respond to the stimuli incident to their presence, with the result that insufficient food material to nourish them is deposited in the cells immediately surrounding the larvae (fig. 4, *A* and *D*). Occasionally the tissues are so hard that the pressure of the enlarging cells within ruptures the epidermis and expels the larva from the plant, as shown in figure 1, *E*. Typical galls of *Harmolita tritici* are shown in figure 1, *F*. The longer galls (at the left) are the forms most commonly observed.

This very important fact was strikingly illustrated in one of the field observation cages in 1933. Females oviposited in two internodes of a culm on the same day. When the larvae were in the second instar, as determined by dissections of other plants of the same cage, the gall tissues at the two infested internodes of this culm were killed in fixing solutions at the same time. These two galls were embedded and sectioned in January 1934. Figure 4, *A*, *B*, *C*, and *D*, shows the gall tissue in the higher internode to be perfectly normal in every way, and the developing larvae are normal. The larvae in the lower, older internode are dead and the gall tissue is not normal. The tissues at this internode were too old and hard when the eggs hatched to respond in the usual manner to the stimuli incident to the presence of the larvae, with the result that sufficient food materials to nourish them were not deposited in the immediately surrounding cells. Thus, the larvae starved not because the plant was not manufacturing sufficient nourishment to supply them, for the larvae located in the tender meristematic tissues at the internode immediately above were then being properly nourished, but because the tissues had become too tough and hard to respond to the stimuli.

Wheat culms may be found having several internodes infested by the jointworm. This is caused either by the emergence of females over several days, or by the survival of individuals for 5 or 6 days. A period of 1 day's growth may serve to render a lower internode unsuitable for oviposition. In the meantime the internode immediately above may become suitable for oviposition. It would be misleading, therefore, to say that any particular internode of the wheat culm is preferred by the female for oviposition. Any internode may be selected in which the meristem is in a stage of development acceptable for oviposition.

It is obvious from the foregoing statements that a delicate balance exists between the period of emergence of *Harmolita tritici* and the development of the meristematic regions of the internodes in the host plant. Plants that are small and medium sized at the time of wheat jointworm emergence are therefore more likely to have their meristematic regions in the stage attractive for oviposition. In a large stem the leaf sheath is thicker, and the vascular bundles lie deeper in the culm, making it more difficult for the insect to reach the vascular tissues in which to place her eggs. It should be practical, therefore, to hasten the growth of wheat in the spring by fertili-

zation, or otherwise, so that the plants would be too far advanced in growth to be in a condition attractive for oviposition when the adults of the jointworm emerge. Failing in that, the advanced stage of growth thus induced would oblige the adults to oviposit so high up in the stem that the galls would be removed with the straw by the reaper at harvest and thus their occupants would no longer be a threat to the succeeding crop. Under average conditions the majority of the galls are so near the ground that they are left in the stubble at harvest.

COMPARISON OF THE MERISTEMATIC REGIONS OF WHEAT PRODUCED IN KANSAS, INDIANA, AND VIRGINIA AS RELATED TO OVIPOSITION

After it was learned that the condition of the meristematic regions of the internodes governed oviposition, it was decided to compare wheat plants from Kansas, where *Harmolita tritici* does not occur, with plants from Indiana and Virginia, where *Harmolita tritici* has caused considerable injury to the wheat crop.

Arrangements were therefore made with the Federal entomological laboratories at Wichita, Kans., and Lafayette, Ind., to preserve whole plants in formalin-alcohol-acetic fixative solution. Wheat plants grown in Virginia were preserved in a similar manner. At 2-day intervals during a period of 18 days, starting a few days before emergence of *Harmolita tritici* adults and ending after the approximate hatching period for the eggs, 50 plants were collected from a field of a variety commonly grown in each locality, and preserved. In Kansas, the time of collection and preservation of stems was based on the emergence of a closely related species, *H. grandis* form *grandis*, which occurs in Indiana and Virginia as well as in Kansas.

The wheat plants from the several localities were examined at the same time at the Charlottesville laboratory, and it seemed, from external appearances, that there were nodes in the proper stage of development for oviposition in all the samples, although the Kansas plants were below the average height and diameter of those from Virginia and Indiana. In this respect it appeared that the Kansas plants might be the more suitable for oviposition. A difference between them and the plants of more eastern origin was found in the firmness of the meristem. This part in the Kansas plants was so firm that it could scarcely be crushed between the forefinger and thumb, whereas in the Indiana and Virginia plants the meristem was spongy and crushed under slight pressure.

Present knowledge of the responses in the tissues of the meristematic regions at the nodes of the wheat plant during gall formation plainly shows that tissues such as those in the Kansas wheat are in a hardened condition and unsuitable for oviposition at the time of emergence of *Harmolita tritici*. Moreover, should the insect succeed in ovipositing in such culms the hatching larvae, in all probability, would not survive.

It is not known at present whether this condition of the meristem in the Kansas plant is due to a varietal or a climatic difference. The main point of interest and importance is that this characteristic undoubtedly makes wheat plants grown in the winter wheat belt of Kansas either unacceptable to *Harmolita tritici* for oviposition, or unsuitable for larval development, or, perhaps, both. If this be true of the Kansas area it is probably true of other sections in the winter wheat belt of the United States where *Harmolita tritici* does not occur.

EFFECT OF OVIPOSITION ON THE PLANT AND SPACING OF THE LARVAE IN THE INTERNODE

The effect of oviposition on the plant tissue is almost negligible, insofar as its normal functions are concerned. The oviposition punctures heal, and scar tissue is found in the path of the ovipositor (fig. 5, *E*). The tissue heals around the eggs, and at the end of a few days the latter appear as inert foreign material embedded in normal tissue (fig. 6, *A* and *D*). Many of the cells immediately above the egg, however, lose their polarity, owing to the disturbance at the time of oviposition (fig. 6, *A*). If the eggs are dissected from the stem just before hatching time there does appear to be the beginning of a gall, although in reality this is simply a cavity in the tissues made by the ovipositor, somewhat larger than the egg, which has completely healed over.

Apparently the normal amount of cell division and other activities proceed as usual after the eggs are placed in the tissues. A few of the cells in the meristematic regions at this time show two nuclei (fig. 6, *B* and *C*). As the cells of the meristematic region divide and increase in length, the eggs, not being deposited in exactly the same horizontal plane, are carried upward various distances in the tissues, and when the larvae are full grown there may be from 1 to 2 inches difference in height between the lowest and highest placed larvae in the same internode (fig. 1, *F*).

SEASONAL HISTORY OF THE GALL

HATCHING AND FEEDING OF FIRST-INSTAR LARVAE

The egg is placed in the tissues with its petiole extending down the stem, and when it hatches the larva also has its head pointing down the stem. The larva apparently ruptures the shell with its delicate mandibles at the union of the petiole and the main body of the egg. The shell is not cast off at once, and the larva feeds head downward, at the point of rupture. From dissections made at this time, it appears that the young larva is bathed in plant sap. It is not definitely known whether some of the surrounding cells are pierced by the mandibles of the first-instar larva, in order to maintain a constant flow of sap, or whether this activity is unnecessary at that time. There is a well-developed digestive tract, even in the first-instar larva, and the greenish, chlorophyll-tinted food in this tract is plainly visible through the body wall. Serial section mounts show that there is no perceptible accumulation of deeply stained matter around the margin of the larval chamber, such as is always present in later instars, indicating that it may not be necessary in the first instar for the larva to puncture the cells in order to obtain sufficient nourishment.

BEGINNING OF GALL FORMATION

By the time the jointworm egg hatches, an uninfested internode of the same stage of development as the infested one has ceased cell division, and normal length and diameter are very soon attained by growth of the cells. When the larva starts feeding, the cells in the immediate vicinity suddenly become very active and increase markedly in size, the nuclei and nucleoli enlarge greatly, the protoplasm becomes very dense, and the walls are thin (fig. 7, *A*). Just beyond this region, which is only 2 or 3 cells deep, the cells begin to divide

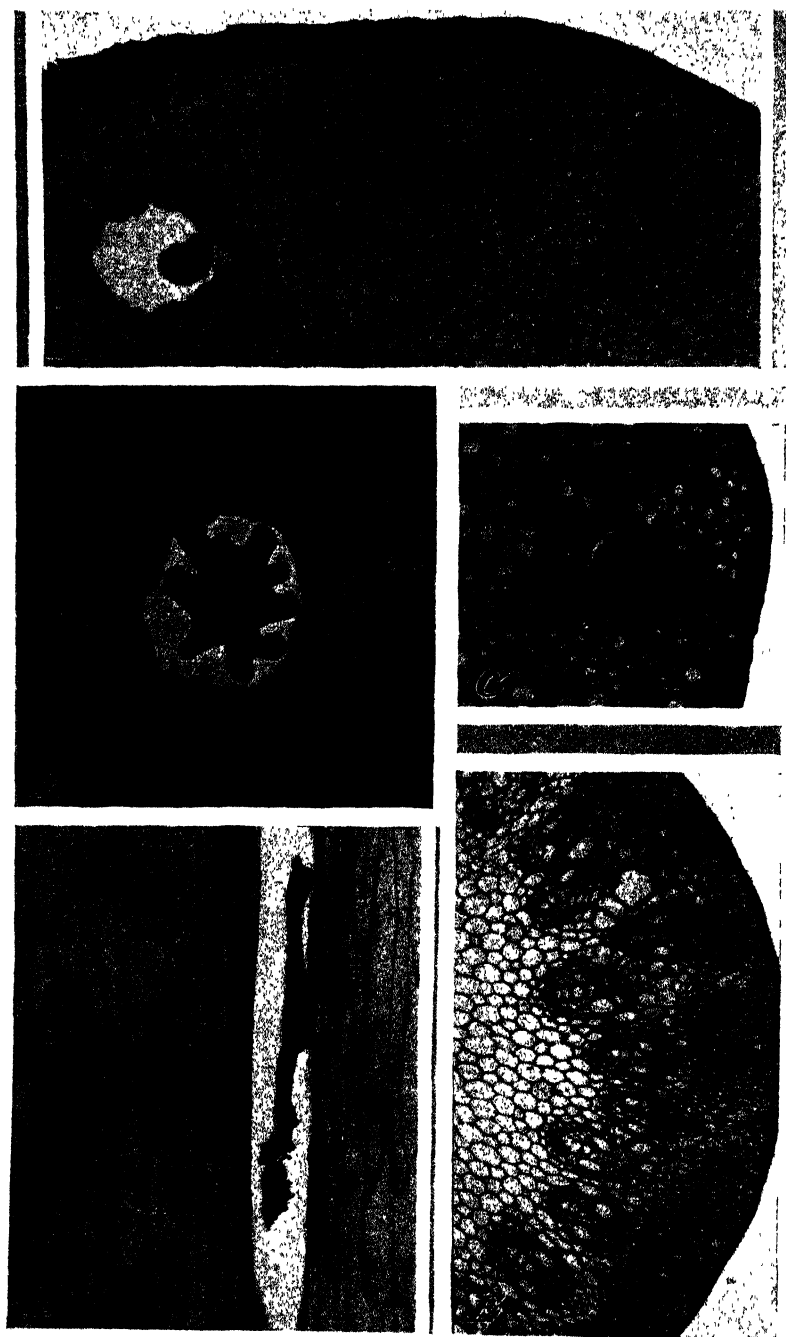


FIGURE 5

(For explanatory legend see opposite page)

(mitotically) rapidly. This change occurs in the vicinity of all larvae, regardless of their position with relation to the meristem. For example, figure 8 shows a larva located a considerable distance up the internode, well out of the active meristematic region, yet cell division is proceeding as rapidly as in the vicinity of larvae located much nearer the node.

Figure 9, *C*, shows recently divided cells lying just outside the area of enlarged cells but immediately surrounding the larva. Note in this figure that these divisions may take place in any plane and that this results in cells of various shapes. Thus, the lack of normal polarity of some cells caused by oviposition (fig. 6, *A*), in addition to the very rapid growth of the cells immediately surrounding the larva, causes the cells in the gall tissue to become irregular both in shape and in arrangement. Figure 9, *B*, shows normal tissue located outside the gall area. Note how regular these cells appear. Figure 9, *A*, shows normal tissue at time of oviposition. Figure 8, *A* and *B*, shows a newly hatched larva, well up the internode, that has induced active cell division. Under normal conditions no further cell division would take place in this region. In other words, the immediate results of hatching and feeding of the young larvae are hypertrophy and hyperplasia, provided the tissues are still plastic enough to respond to the stimuli. Where the tissues are too old, the result is simply hypertrophy, and the larva starves and eventually dies (fig. 4, *A* and *D*), since the tissues are then unable to deposit sufficient food materials to nourish it. It is a fact well known to students of gall tissues, that a gall-producing larva cannot stimulate old tissues into activity.

ACTIVITY DURING THE SECOND INSTAR

Before the second instar is reached, cell division ceases and all later growth is by simple hypertrophy. The cells in the immediate vicinity of the second-instar larva become very much larger than in the case of the preceding instar (fig. 7, *A* and *B*), and the cells associated with succeeding instars are progressively larger (fig. 7, *C*). Figure 10, *A*, shows some of the cells in the food-tissue zone of the third instar more highly magnified to illustrate the density of the protoplasm. By examining figure 10, *E*, the great contrast between the gall tissue and normal tissue will be seen. The nuclei and protoplasm have the same general appearance, except that the nuclei and nucleoli are very much larger and the nucleoli are of irregular shapes in later instars (fig. 10, *B*, *C*, *D*). These cells are what the European writers on insect galls have called "food tissue." There is no definite number of layers of such cells about a larva nor are they arranged in any particular direction as in some of the cynipid galls; they have lost their normal arrangement and point in various directions (fig. 4, *F*), although the general trend of the long axes of the cells is vertical (fig. 7, *A* and *B*).

EXPLANATORY LEGEND FOR FIGURE 5

A, Part of a transverse section that contained a normal second-instar larva and an unhatched egg, $\times 75$. Egg may be seen in the upper right corner. *B*, Unhatched egg highly magnified ($\times 400$), showing that the egg is surrounded by parenchymatous tissue, whereas the larva shown in *A* has the normal gall tissue developed about it. *C*, Unhatched egg in part of a transverse section in which normal third-instar larvae were also present. Note that the parenchymatous tissue surrounding this unhatched egg is now thick-walled and lignified, $\times 112$. *D*, Part of a longitudinal section containing a dead second-instar larva. Note that the cells surrounding the larva have changed from the normal food tissue cells to the parenchymatous type, $\times 100$. *E*, Scar tissue developed in the path of the ovipositor, 9 days after oviposition. No gall tissue has developed here, $\times 64$.



FIGURE 6.—A, Longitudinal section through an egg 7 days old. Note that the cells above the egg have lost their normal polarity, $\times 180$. B and C, Cell with two nuclei from tissue containing eggs 9 days old; B, $\times 1,050$; C, $\times 750$. D, Cross section through an egg 9 days old. Note that the plant cells have closed the cavity about the egg, and the vascular bundle in which it was originally placed no longer recognizable, $\times 115$.

Every vascular bundle that contains a larva is entirely disintegrated. In case the internode contains as many as 15 or 16 larvae, the vascular tissue will have almost entirely disappeared. The vascular tissue that may be found in such gall tissue consists of a few irregular bundles, all of which may be pushed to one side of the stem (fig. 11, *A* and *B*). These bundles are not parts of vascular tissue originating in the new gall tissue. Therefore, practically all food materials that pass through this area must apparently be largely transferred through the gall-tissue cells, as the normal vascular system no longer exists. Naturally this derangement in the vascular system is a great hindrance to the plant and a serious drain upon its resources, as is indicated by the fact that the yield from infested plants is much less than from normal plants of similar size.

As practically all of the vascular bundles are destroyed in the infested portions of the internodes, the culms become very weak at these points, and consequently a windstorm or heavy rain will cause the infested culms to fall or lodge. This will occur only during the first or second instar of the insect, since in the later instars the gall has a large amount of modified supporting tissue developing in it (fig. 12, *B*). Frequently the weight of the culm alone will cause the internode to loop (fig. 1, *F*) in the gall area where the vascular bundles have been destroyed. The heads and galls of fallen or lodged plants frequently escape the harvester. This permits the galls to remain as a source of infestation in the following season.

By the time the larvae have reached the second instar, the internode within the gall area is usually completely filled with thin-walled, parenchymatous gall tissue. This gall tissue arises mainly from the parenchyma, but partly from the phloem, during the first larval instar. No further cell division was observed during the later instars, the gall tissue increasing in volume by hypertrophy.

During the second instar the walls of the cells bordering the so-called "nutritive zone" (toward the center of the stem) begin to thicken and show pits (fig. 12, *A*). Lignification begins at this time. The condition of the nuclei and cytoplasm of these cells indicates that they are active, living cells.

As previously stated, when the larva breaks through the eggshell its head points down the stem. In the course of the second instar it reverses its position within the larval cell, and in all following instars its head points up the stem.

ACTIVITY DURING LATE INSTARS

As the larva grows older its mandibles become larger and more heavily chitinized and sclerotized with each molt, and it continues to puncture cells of the food tissue and to devour their contents. During the first and early second instars the main difference in the cells in the gall area is that the cells in the immediate vicinity of the larva are larger and more heavily charged with food material. All the gall cells are thin-walled at this time. The cavity occupied by the larva increases in dimensions through the destruction of the cells of the food tissue surrounding it, and the size of the larval cavity apparently keeps pace with the requirements of the growing larva. There is always a deep layer of rich food tissue about the larva regardless of the number of cells destroyed for food, and a constant supply of such

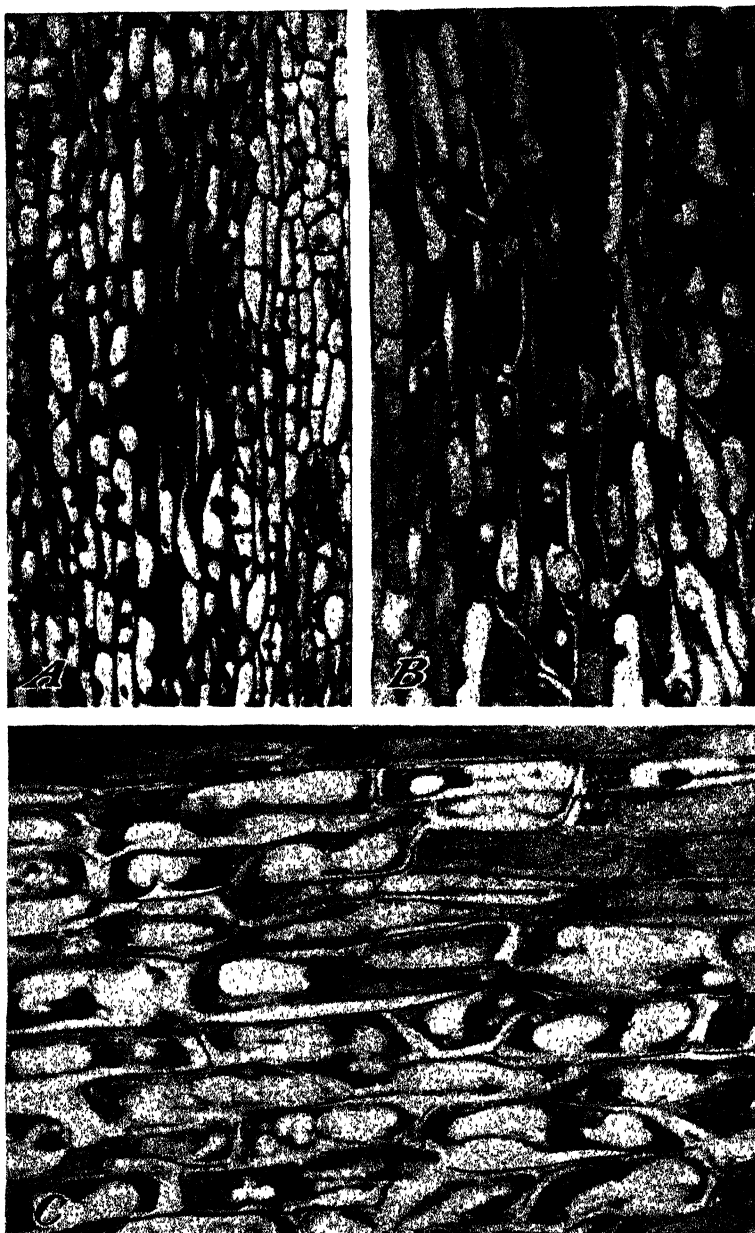


FIGURE 7.—*A*, Gall tissue near first-instar larva; *B*, gall tissue near second-instar larva; *C*, gall tissue near third-instar larva. These photomicrographs illustrate how the gall tissue increases in volume by hypertrophy; all $\times 100$.

tissue is maintained by the enlargement of the cells already present. Cell division is not a factor in the maintenance of this food tissue.

As the larva and plant increase in age the condition of the cells shown in figure 12, *A*, becomes more pronounced (fig. 12, *C*). These



FIGURE 8.—*A*, Longitudinal section through an internode containing an early first-instar larva that was feeding but had not cast off the entire eggshell. The larva is the long, dark object on the right near the top. The wall of the internode is thicker at this point and the larva is quite a distance above the node, $\times 26.4$. *B*, Mitotic cell division in the vicinity of the larva. This is the stage when hypertrophy and hyperplasia occur at the same time, $\times 850$.

cells originate from the parenchymatous gall tissue, starting near the center of the stem and from those cells farthest from the larva (fig. 12, *B*). This lignifying tissue advances progressively toward the larval cavity until the food tissue is only 3 or 4 cells deep by the time the larva becomes full grown (fig. 11, *B*). Very shortly after the larva

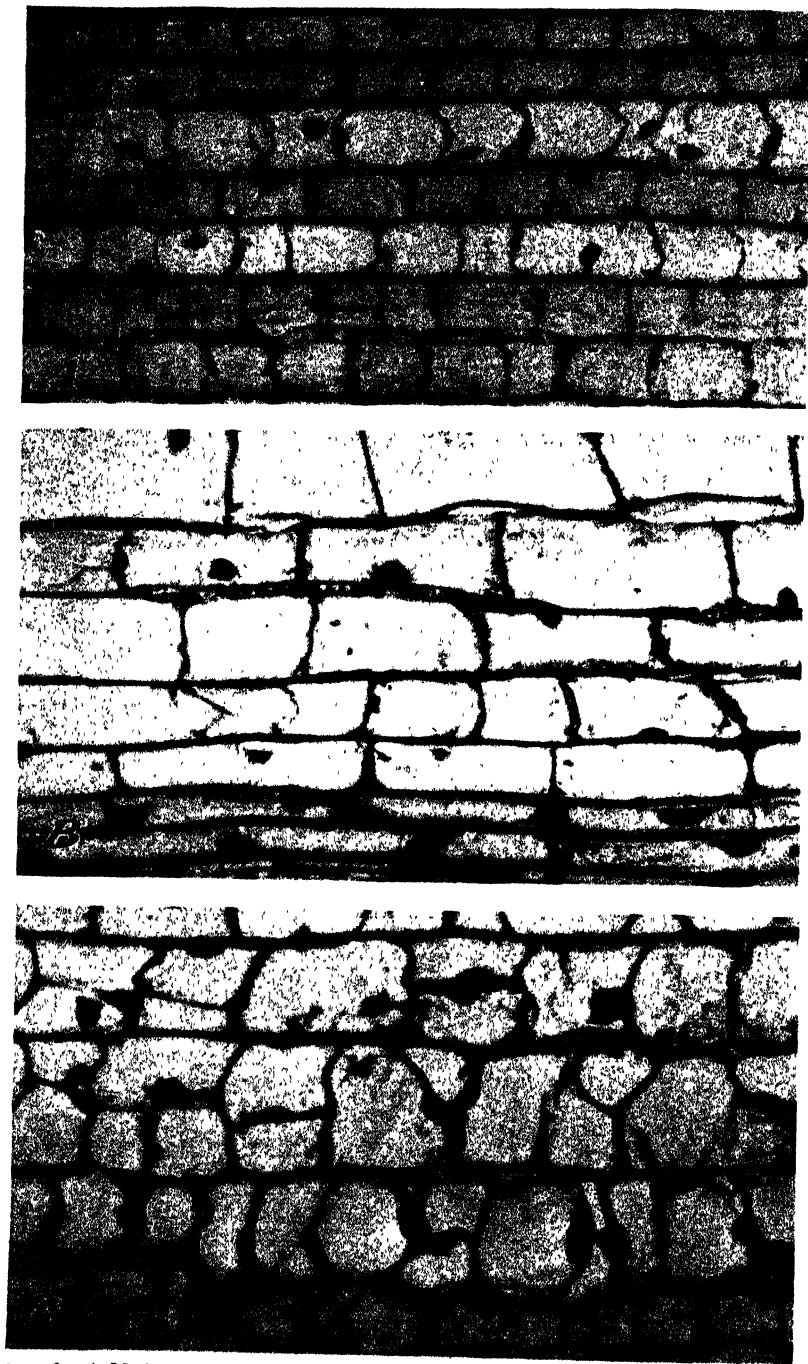


FIGURE 9.—*A*, Meristematic tissue at the time eggs are deposited, $\times 220$; *B*, normal parenchymatous tissue outside the gall area in an internode with first-instar larvae, $\times 220$; *C*, cells in the gall area near a first-instar larva, $\times 250$.

stops feeding, all the tissue surrounding the larval chamber becomes lignified, and by the time the wheat is harvested this lignified gall tissue, with the exception of a thin layer around the larval chamber, has become as hard as white oak wood, instead of being in the normal condition of an unfested stem as shown in figure 13.

The larva always maintains a very thin wall of cells between itself and the outside of the stem, and the full-grown larva is always found with the ventral surface of its body toward the outside of the stem. When, therefore, the adult casts off the pupal skin, the insect is ready to gnaw its way out through a very thin wall.

There is no indication that fecal matter is discharged into the larval cell or chamber at any time during growth. The larva changes to a prepupa in November and December, and the fecal matter is discharged at that time.

About a month elapses from the time the eggs are deposited until the larvae become full grown. The eggs hatch in about 12 days, which is almost half of this entire period. The first and second instars require about 5 days each, making 22 days from oviposition to the beginning of the third instar, and the remaining instars are completed in about 8 days.

It is interesting to note that this acceleration in development coincides with the rapid maturing of the wheat plant. If the last instars required as long as the first two, the larval period would then be carried beyond the maturity of the plant, and the larva would perish from lack of nourishment.

MICROCHEMICAL TESTS

As a matter of interest, a number of chemical tests were made of the gall tissue in serial sections on slides, according to Zimmermann's technic (12). Tests made for the presence of tannin and starch gave negative results; those for protein, cellulose, and lignin were positive. The contents of the cells in the food-tissue zone gave the positive test for protein, the cell walls in this zone gave the positive test for cellulose, and the thick-walled cells in the more mature gall tissue reacted to the lignin test.

ZONES IN GALL TISSUE

European and North American students of gall tissue state that several fundamental zones of tissue are found in insect galls, although they do not agree on the exact number. Cosens (2, p. 354) states that the cynipid galls have 3 or 4 zones, mostly 4; Küster (7) found 4; Weidel (11) and Ross (9) illustrated 4; Kinsey (6, p. 40) states there are, with a few exceptions, 5. According to Kinsey's interpretation, both Weidel's and Ross' illustrations may be construed as having 5 zones.

The wheat jointworm galls form within the internodes of the culms. Wheat stems do not have as many different kinds of tissue as do the leaves and twig tips of oaks; nor have the *Harmolita* galls as many tissue zones as the cynipid galls. Indeed, there are no sharply defined concentric tissue zones in the galls of *H. tritici* as have been described and illustrated for the cynipid galls. The gall tissues in the former arise partly from phloem, but mainly from the



FIGURE 10.—A, Cells in the food tissue near the third-instar larva, highly magnified ($\times 275$) to show the dense protoplasm and large nuclei. B, C, and D illustrate the peculiar shape of the nucleoli of some of the cells in the nutritive zone, $\times 530$. E, Normal parenchymatous cells outside the gall area of third-instar larvae, $\times 530$.

parenchyma, and fill the pith cavity. As is shown in the longest segment in figure 1, *F*, there is usually little external evidence of galls in wheat stems.

Ross' figure (9) of the gall on *Ficus* indicates that *Harmolita* galls on such plants are very similar to cynipid galls.

There are apparently no other galls mentioned in literature similar to jointworm galls on wheat stems. Most insect galls usually involve the leaves, petioles, veins, or leaf buds, whereas the wheat jointworm galls involve the main stem of the plant. There is no pith cavity in the gall area of the internode, the entire stem at this point being filled with gall tissue. There may be as much as 2 inches of the internode thus involved and, occasionally, in heavy infestations, 2 or 3 internodes may be infested. It is obvious, therefore, how seriously this gall tissue affects the normal functions of the plant.

The epidermis and sclerenchyma are changed very little during the process of gall formation, although these cells later become very thick walled and lignify (fig. 11, *A* and *B*). The parenchymatous cells immediately surrounding the larval chamber become heavily charged with very dense protoplasm and form what is known as the nutritive zone. There does not seem to be any definite number of cell layers to this zone, and there is no definite arrangement of these cells; in fact, a large number of the cells in the gall area lose their polarity as shown in figure 7, *A* and *B*, although the general trend is vertical. In the later larval instars these parenchymatous cells become thick walled, pitted (fig. 12, *A* and *B*), and lignified. The sclerenchymatous cells, just below the epidermis, and the epidermal cells also become thick walled and lignified. In the earlier instars of the insect, between the larval chamber and the epidermis are found the nutritive, the parenchymatous, and the sclerenchymatous zones. In other words, according to Kinsey's nomenclature, there are in the second larval instar 4 zones from the outside of the stem to the larval chamber, and 3 zones from the larval chamber to the center of the stem (fig. 11, *A*). When the gall tissue and the wheat plant are thoroughly mature there appear to be two zones of lignified tissue—a hard zone immediately surrounding the larva, and the remainder of the gall, which is very much harder.

THE GALL STIMULUS

Beyerinck (1) has stated that the initial stimulus of gall formation is a secretion from the poison glands introduced into the tissues with the ovipositor, and has figured a well-developed gall in which neither egg nor larva was found. Magnus (8) has stated that the secretion of the poison glands was without significance; that the start of gall formation was coincidental with the wounding of the tissue in oviposition; that the second impetus came with the feeding of the larva; and, further, that the gall tissue ceased development with the death of the larva. Cosens (2, p. 371) has stated that the larva secretes an enzyme that is the active agent in gall formation. Kinsey (6, p. 37) believes that the precise source and nature of the gall-producing stimulus are not known but thinks "it partakes of the nature of an enzyme or hormone produced by some particular insect structure."

Magnus (8) terms "Galligenes Organ" the short appendage at the posterior pole of the egg of the *Harmolita* from galls on *Ficus* spp. He



FIGURE 11.—A, Part of a transverse section through the gall tissue around a second-instar larva, illustrating the lack of vascular tissue, the food-tissue zone with the cells filled with rich food material, the thick-walled cells developing deeper in the gall tissue, and the manner in which the epidermal and the sclerenchymatous cells have been modified, $\times 50$; B, part of a transverse section through the gall tissue at the time the larva is full grown, $\times 36$. Note that practically all the tissue has lignified except the thin layer of food tissue around the larva. Note also that there are only a few small vascular bundles present.

suggests that a gall-forming irritant may pass out through it from the egg into the surrounding tissues. Magnus finds that several colloidal stains will readily pass into this appendage and diffuse to other parts of the egg. He also states that a large amount of gall tissue forms before the egg hatches. Ross (9) says that little is known about the gall-making irritant (Reizwirkung); that there are different kinds, some chemical, which arise mostly in the salivary glands and function as a kind of enzyme. Ross further states that there is a wound irritant (Wundreiz) that plays an important part in gall formation.

Apparently no gall-producing stimulus is introduced into the plant tissues at the time of oviposition by *Harmolita tritici*. This is clearly indicated by the fact that unhatched eggs about which no gall tissue had formed were found in tissues near second- and third-instar larvae (fig. 5, A, B, and C), whereas normal gall tissue had formed about the larvae. Furthermore, the condition of the cells in the vicinity of eggs 9 days old and of those in the vicinity of the eggs just mentioned appears to be the same (figs. 5, B; 6, D). As soon as the eggs hatch, gall tissue forms rapidly. Should the larva die for some cause after the gall tissue had formed about it, the food tissue ceases to function as such (fig. 5, D) and becomes modified parenchymatous tissue. It is obvious, therefore, that the presence of a living larva is necessary for the formation and continuance of gall tissue.

Smith (10, pp. 177, 183, 184) has shown that there are many substances, both alkaline and acid, that will cause proliferation of cells when introduced into the tissues of plants at the proper dilution. He says:

From these experiments there can be no reasonable doubt, I think, that any soluble substance whatever, except a killing, a plasmolyzing or an oxygen-absorbing substance, if continually liberated in excess locally in tissues, would be competent to induce tumor formation.

He also says:

* * * I think I have established my original hypothesis, viz, that dilute ammonia causes intumescences and have rendered it probable that ammonia liberated within the cell in small quantities by the imprisoned bacteria must be one of the causes of the excessive and abnormal cell proliferation in crown gall. Probably amin compounds also help to determine it. Since an acid and alcohol are likewise produced by the crown gall bacteria and this alcohol and this acid (as well as many other acids) in pure dilution and also in combination with ammonia caused galls or intumescences in my experiments, the acid (or acids), the alkalies, and the alcohol must, I believe, act together in producing the tumors, and osmotically rather than chemically. * * *

To conclude, it would seem, therefore, that in local osmotic action (possibly in some stages chemical action also) of various substances (aldehyde, acetone, alcohol, acids, alkalies) thrown into cells and diffusing from them in various directions, as the result of the metabolism of a feeble intracellular parasite or symbiont together with the resultant countermovements of water and food supply we have, in crown gall at least and presumptively also in animal neoplasms, the explanation of tumor growth—that is, of that extensive multiplication of cells in opposition to physiological control which has so long puzzled pathologists and all students of overgrowths.

If the byproducts of metabolism of the crown gall organism, and various acids and alkalies, can cause tumor growths in plants, there would seem to be no valid reason why the byproducts of metabolism of larvae alone, when continuously liberated in plant tissues, would not produce galls. It also seems probable that irritation resulting from the larvae puncturing the cells of the food tissue might be a contributing cause.

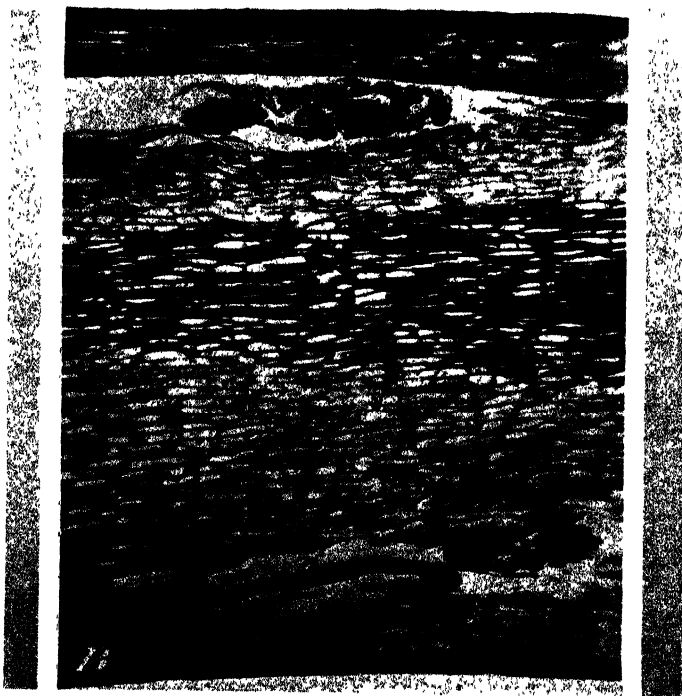
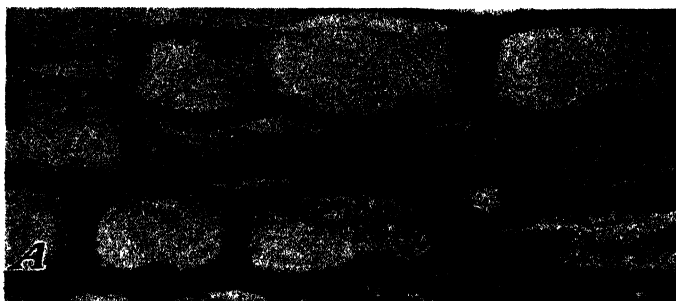


FIGURE 12.—*A*, Part of a longitudinal section through gall tissue around second-instar larvae; note that this tissue is becoming thick-walled, and pits are present in the walls, $\times 400$. *B*, Part of a longitudinal section through gall tissue around third-instar larvae, a part of the tissue between the larvae has become thick-walled and is lignifying, $\times 25$. *C*, a few of the thick-walled cells shown in *B*, highly magnified to show the thick walls and the numerous large pits, $\times 400$.

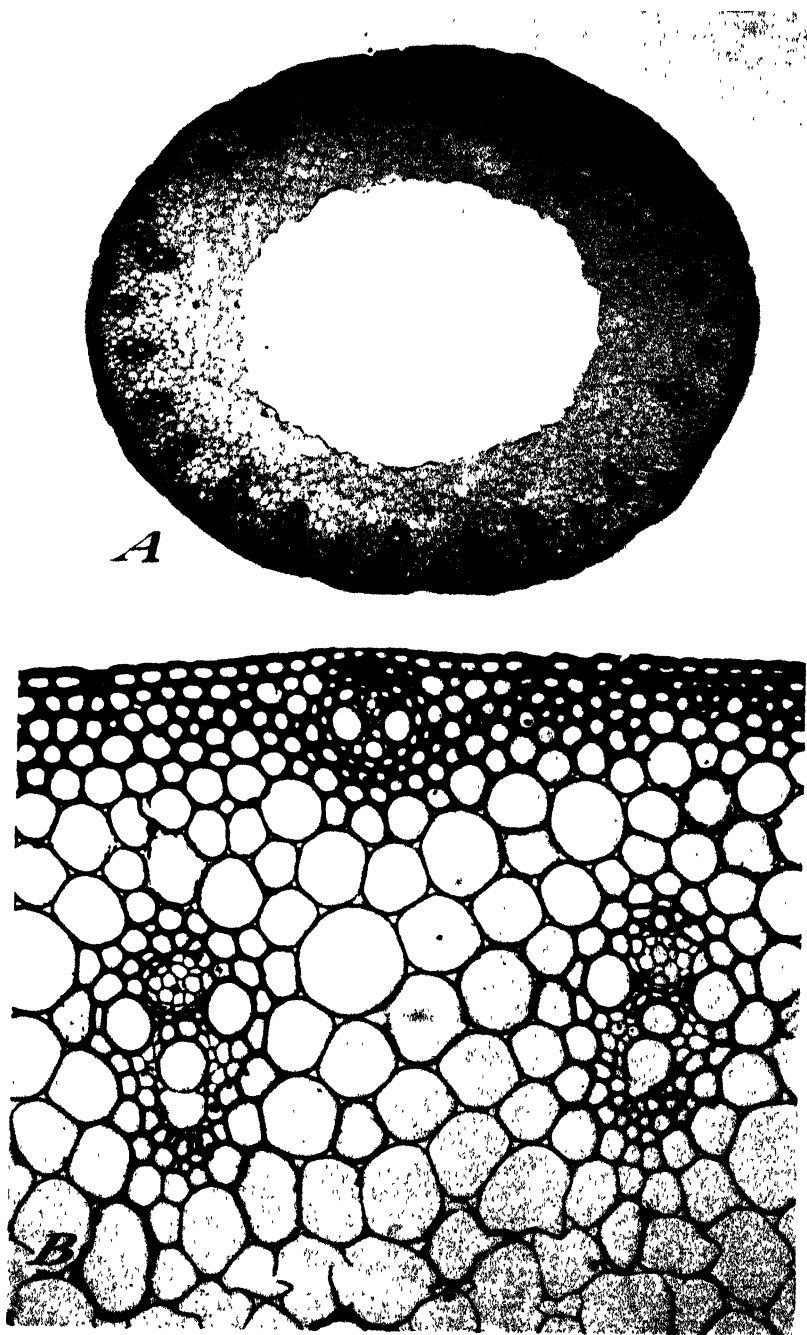


FIGURE 13.—*A*, Transverse section of a normal internode of a wheat culm at the time the jointworm larvae would be full grown, $\times 20$. Note the number, the size, and the regular spacing of the vascular bundles as compared with those in gall tissue illustrated in figures 4 and 11. *B*, A part of the section represented in *A*, $\times 170$.

DISCUSSION

Entomologists have speculated for years as to the reason why *Harmolita tritici* does not occur west of central Missouri and in the Great Plains area. This insect has been known as a serious pest since 1848, and there has been ample opportunity for it to spread into this territory, either by natural means or through the avenues of commerce. The writers believe that this apparently strange phenomenon is explained by the facts presented in this paper. The explanation is very simple. Either the meristematic regions of the wheat culms at the oviposition period are too hard and tough to be acceptable for oviposition in these uninfested areas, or, should oviposition occur, the larvae fail to reach maturity through causes explained in the foregoing discussion.

It is not known at present whether the early hardening of the meristematic region of the internodes in the Kansas wheat plants is due to climatic or varietal differences. If it is varietal, this character could perhaps be transferred to varieties grown in the Eastern and Central States. However, whether this be practical or not, it is known that the insect requires very soft meristematic tissue for oviposition. Therefore, it should be possible through fertilization, or otherwise, to force the growth of the wheat beyond the attractive stage before the adults of the jointworm emerge. Failing in that, it should be practical so to accelerate the growth that oviposition will occur high up the culm. The galls would then be removed by the reaper at harvest and the succeeding infestation thus greatly reduced.

SUMMARY

A study of the gall development in wheat stems following attack by *Harmolita tritici* indicates that the absence of the insect from the Kansas and western Missouri wheat fields is due to the character of the stem in wheat grown in this immense district.

The female locates the vascular bundle in the stem just above an unexposed node by probing for the xylem tubes, and places but one egg in any vascular bundle.

It is difficult for the lancelike ovipositor to pierce the leaf sheath and stem of the wheat plant, consequently the female usually refuses to oviposit in any except the very delicate tissue in the meristematic region just above the node. The larvae do not survive when the egg is placed in older and tougher tissue.

As many as 5 or 6 eggs may be deposited through a single external oviposition puncture, and three such punctures may be made at the same node in practically the same horizontal plane. Eggs are placed only in or near the phloem of the vascular bundle. This is the only gall-making insect that the writers know to have this habit.

The most favorable temperature for oviposition is about 80° F. Under the most favorable conditions the females will deposit the majority of their eggs and die at the end of about 2 days after emergence.

In the Kansas wheat it was found that the meristematic region at the node thought to be susceptible to oviposition was so tough and hard as, doubtless, to be unsuitable. Should oviposition occur in such tissue, the cells would not respond to the stimuli essential for

the development of the normal gall tissue necessary for the nourishment of the larvae of *Harmolita tritici*. This explains why this insect does not occur in the Kansas wheat belt and, perhaps, in certain parts of central Missouri.

Unhatched eggs remain as inert foreign substance embedded in the tissue. This strongly indicates that the female does not inject a stimulating substance into the tissues at the time of oviposition to initiate the production of gall tissue.

Hyperplasia and hypertrophy start at once upon hatching of the eggs, and cell multiplication is by mitosis only. Hyperplasia ceases after the first instar, and is succeeded by hypertrophy. Gall tissue arises partly from the phloem but mainly from the parenchyma, and the cells in the gall area lose their normal polarity. There is no pith cavity in the part of the internode within the gall area, this section of the internode being filled with gall cells. Practically all the vascular tissue is destroyed in this region also, and this greatly interferes with the normal functions of the plant. There are no concentric layers or zones in the gall tissue as is common in the cynipid galls. The entire region lignifies and becomes hard by the time the larvae are full grown.

The gall stimulus is apparently due to byproducts of larval metabolism and, perhaps, to mechanical irritation.

It should be possible to so accelerate the growth of wheat in the early spring, by fertilization or otherwise, that the plants would be unacceptable for oviposition or unsuitable for larval development at the time of attack. Even if this failed, the additional growth caused by such measures would cause the galls to be located so high up on the culms that they would be removed by the reaper at harvest and thus greatly reduce infestation in the following crop.

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EFFECT OF CROWN RUST INFECTION ON YIELD AND WATER REQUIREMENT OF OATS¹

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INTRODUCTION

Crown rust (*Puccinia coronata avenae* Eriks.), the most generally prevalent rust of oats (*Avena sativa* L.), is virtually coextensive with oat culture. Estimated annual losses caused by the rust in the United States from 1918 to 1930, inclusive, averaged more than 13,700,000 bushels. Immer and Stevenson (3)³ and Immer and Ausemus (2) have shown that severity of crown rust infection is negatively correlated with yield, plumpness, date of heading, and height of oats. Weaver (11) stated that the water requirement

$$\left(\frac{\text{weight of total water transpired}}{\text{total dry weight}} \right)$$

of seedling oat plants bearing an infection of 0.5 percent of stem rust (*P. graminis avenae* Eriks. and Henn.) on the first leaf was 37 percent greater than that of nonrusted plants. His observations were limited to a period of 7 days and included only 8 diseased and 9 control plants. He used crown rust in an earlier experiment extending over a period of 153 hours and found the transpiration of the seedling plants with crown rust on the first leaf to be 94 percent greater than that of similar nonrusted plants.

The effect of rust infection on the water requirement of other cereals has been reported by only a few investigators. Weiss (12) studied the effect of stem rust and leaf rust infection on the yield and water requirement of Marquis wheat. He found that rusted plants had a higher water requirement than nonrusted ones, although the differences were significant only in the case of stem rust infection. Infection with either rust caused a significant reduction in yield of tops and of grain, the yield of the latter being most affected. Weaver (11) studied rust-infected wheat, rye, barley, and corn, in addition to oats, and found that the transpiration rate was consistently higher in the rusted plants. Johnston and Miller (4) have published a preliminary report of their studies on the effect of leaf rust infection on water economy and growth of wheat. They found that the water requirement of susceptible varieties increased with increasing length of association of host and parasite, while the yield of all plant parts was materially reduced.

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³ Reference is made by number (italic) to Literature Cited, p. 410.

The experiments reported in this paper were planned as a more extensive investigation of the relation of crown rust infection to yield and water requirement of resistant and susceptible oat varieties. Data are presented that give information concerning (1) the relation of the total duration of crown rust infection to reduction in yield of a susceptible variety growing under field conditions, (2) the relation of the total duration of infection to yield and water requirement of a susceptible pure line and a resistant one growing under greenhouse conditions, and (3) the interrelations of soil moisture and time of initial infection with yield and water requirement of 2 susceptible lines and 1 resistant pure line under greenhouse conditions. A preliminary report of this work has been published by the writer (8).

EFFECT OF CROWN RUST INFECTION ON YIELD OF FIELD-GROWN OATS

MATERIAL AND METHODS

An investigation was conducted in the summer of 1928 to determine the relation between the total duration of crown rust infection and the subsequent reduction in yield of a susceptible oat variety growing under field conditions. A pure-line selection of Iomine oats (C. I.⁴ no. 2827) was sown uniformly on April 12 in 30 consecutive plots of 10-rod rows (one two-hundred-and-sixty-fourth of an acre) each at Ames, Iowa. The plots were spaced 3 feet apart, while the rod rows within the plot were 1 foot apart. Soon after the seed was sown, a heavy rain partly eroded plots 7 and 21 and they were later omitted from the experiment. The plants in 15 of the 28 plots were dusted on certain dates with sulphur at the rate of 15 pounds per acre, while those in 8 of the remaining 13 plots were artificially inoculated with crown rust at definite intervals. Urediospores were obtained in abundance from heavily rusted greenhouse plants and dusted on the moistened plants soon after sundown. The number and arrangement of the plots in relation to the treatment given are shown in table 1.

RESULTS

The year 1928 was very nearly ideal for a study of the effect of crown rust infection on yield of field-grown oats at Ames, because there was an almost complete absence of natural dissemination of either crown rust or stem rust. There was, however, a considerable spread of crown rust from artificially inoculated plants to nearby plots. Stem rust was almost entirely absent, not more than a trace being observed in any of the oat plots.

The kinds and dates of treatment, percentage of crown rust infection on July 5, bushel weight, and acre yield for each of the 28 plots of Iomine oats grown in 1928 are given in table 1. Dusting with sulphur did not entirely prevent the incidence of crown rust, although it was remarkably effective, considering the lateness and the small number of applications. The spread of crown rust from the plots first artificially inoculated was evident. The percentage of infection in each of the plots was recorded on July 5, when crown rust apparently had attained its maximum intensity in all of the plots, although it had appeared in the artificially inoculated ones in great abundance 7 or 8 days after the first inoculation. The sulphur-dusted plants then averaged soft- to hard-dough stage of development.

⁴ C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

TABLE 1.—Effect of crown rust infection on bushel weight and acre yield of 28 plots of Iomine oats grown at Ames, Iowa, in 1928

Plot no.	Treatment	Dates of treatment	Percentage of infection on July 6 ¹	Weight per bushel	Yield per acre	Difference between rusted plot and average of 2 adjacent plots	
						Weight per bushel	Yield per acre
		<i>June</i>		<i>Pounds</i>	<i>Bushels</i>	<i>Pounds</i>	<i>Bushels</i>
1	Dusted with sulphur	24, 27, 29	10	28.0	65.0		
2	Artificially inoculated ²	9, 16, 22, 29	100	20.6	33.2	-6.05	-34.05
3	Dusted with sulphur	24, 27, 29	15	25.3	69.5		
4	Artificially inoculated ²	9, 16, 22, 29	100	19.3	23.4	-5.35	-40.05
5	Dusted with sulphur	24, 27, 29	10	24.0	57.4		
6	Artificially inoculated ²	16, 22, 29	100	21.6	44.6	-3.40	-24.50
8	Dusted with sulphur	24, 27, 29	10	26.0	80.8		
9	Artificially inoculated ²	16, 22, 29	100	23.0	57.2	-4.00	-23.75
10	Dusted with sulphur	24, 27, 29	5	28.0	81.1		
11	Artificially inoculated ²	22, 29	100	23.6	54.4	-3.40	-21.60
12	Dusted with sulphur	24, 27, 29	5	26.0	70.9		
13	Artificially inoculated ²	22, 29	100	22.6	40.3	-3.20	-20.80
14	Dusted with sulphur	24, 27, 29	3	25.6	51.3		
15	Artificially inoculated ²	29	100	23.0	31.1	-2.80	-29.40
16	Dusted with sulphur	24, 29	3 1/2	26.0	69.7		
17	Artificially inoculated ²	29	100	25.0	45.7	-2.80	-27.80
18	Dusted with sulphur	21, 29	1+	29.6	77.3		
19	None		45	26.6	60.4	-2.50	-15.55
20	Dusted with sulphur	24, 29	1	28.6	74.6		
22	do	24, 29	1	26.6	66.3		
23	None		30	25.0	42.9	-2.10	-16.90
24	Dusted with sulphur	24, 29	1	27.6	53.3		
25	None		20	24.6	38.7	-2.50	-10.05
26	Dusted with sulphur	24, 29	1-	26.6	44.2		
27	None		15	25.0	35.4	-2.10	-10.00
28	Dusted with sulphur	24, 29	1-	27.6	47.8		
29	None		10	24.0	45.0	-2.60	-7.10
30	Dusted with sulphur	24, 29	1-	25.6	56.4		

¹ The percentage severity of rust infection was determined by comparison with the scale for estimating rust percentages adopted by the Division of Cereal Crops and Diseases.

² None of the artificially inoculated plots was sulphur-dusted.

³ Trace of infection is indicated by t.

The plants first inoculated with crown rust on June 9 were in full head about 5 days later than those in the adjoining sulphur-dusted plots. These same rusted plots appeared to ripen about 4 days earlier than the adjoining sulphur-dusted ones. The ripening was premature, however, and was doubtless caused by the increased susceptibility of the heavily rusted plants to the very hot, dry weather. The other artificially inoculated plots were affected similarly but to a less degree.

The differences in both acre yield and weight per bushel between the plots heavily infected with crown rust and the adjoining sulphur-dusted plots are apparently highly significant in every case. The data presented in table 1 may be summarized by calculating the average weight per bushel and the yield of the replicated treatments. Because of the presence of a considerable degree of soil heterogeneity, it seems preferable to compare the replicated rusted plots with the immediately adjoining sulphur-dusted ones only. The average weight per bushel and yield of the various replications and the percentage weight and yield in comparison with those of the immediately adjoining sulphur-dusted plots are presented in table 2.

TABLE 2.—*Effect of crown rust infection on bushel weight and acre yield of certain plots of Iomine oats grown at Ames, Iowa, in 1928*

[Values are averages of plots treated]

Plot no.	Treatment	Dates of treatment	Infection on July 5	Weight per bushel		Yield per acre	
		June	Percent	Pounds	Percent	Bushels	Percent
1, 3, 5	Dusted with sulphur	24, 27, 29	11.7	25.77	100.0	63.97	100.0
2, 4	Artificially inoculated	9, 16, 22, 29	100.0	19.95	77.4	28.30	44.2
5, 8, 10	Dusted with sulphur	24, 27, 29	8.3	26.00	100.0	73.10	100.0
6, 9	Artificially inoculated	16, 22, 29	100.0	22.30	85.8	50.90	69.6
10, 12, 14	Dusted with sulphur	24, 27, 29	4.3	26.53	100.0	67.77	100.0
11, 13	Artificially inoculated	22, 29	100.0	23.10	87.1	47.35	69.9
14, 16, 18	Dusted with sulphur	24, 29	1.2	27.07	100.0	66.10	100.0
15, 17	Artificially inoculated	29	100.0	24.00	88.7	38.40	58.1
18, 20, 22, 24, 26, 28, 30	Dusted with sulphur	24, 29	.2	27.46	100.0	59.99	100.0
10, 23, 25, 27, 29	None		24.0	25.04	91.2	44.48	74.1

Plot 14 was dusted with sulphur June 24, 27, and 29

The greatest decrease in bushel weight and in yield per acre was observed in those plots first artificially inoculated on June 9. These plots yielded 44.2 percent as much as the adjoining sulphur-dusted ones, and the bushel weight of the grain from the artificially inoculated plots was only 77.4 percent as much as that of the grain from the sulphur-dusted check plots. The decrease doubtless would have been even greater had the sulphur dusting entirely prevented the appearance of rust in the check plots. Those plots in which the initial inoculation was made on June 16, a week later, showed a much smaller reduction in bushel weight and yield. There was no significant difference in reduction in yield between the replications initially inoculated on June 16 and June 22, respectively. There was, however, an apparently significant difference in the bushel weights of the two treatments. The reduction in yield of the plots inoculated June 29 was greater than that of those inoculated June 16 and 22, although the reduction in bushel weight was smaller. The fact that the former were dusted with double the quantity of spores used for the others inoculated on the same date may account in part for the unexpectedly greater reduction in yield, although on July 5 there was no apparent difference in the degree of infection in any of the artificially inoculated plots. The secondary infection in plots 2, 4, 6, and 9 was apparently equal to that resulting from the extra inoculum used on plots 15 and 17. There was sufficient spread of rust from the artificially inoculated plots to produce an average of 24 percent infection in the five untreated plots. This apparently reduced the yield approximately one-fourth, while the percentage weight of the bushel was 8.8 less.

It is evident that there is a definite relation between duration of rust infection and bushel-weight reduction. Apparently the earlier the incidence of infection the greater will be the reduction in weight. This relationship, no doubt, would have been even more evident had the sulphur applications been made early and frequently enough to prevent the appearance of any crown rust in the check plots. No such exact indication of relationship appeared between the time of initial infection and yield, although, with the exception of replicated plots 15 and 17, there appears to be a similar relationship. The

reduction in yield in these two plots is greater than would be expected on the basis of the bushel weight and from the results obtained later from greenhouse experiments.

There was no indication that sulphur in itself significantly affected the yield. In an experiment conducted on the agronomy farm by the Soils Department of the Iowa State College, the application of sulphur to the soil did not result in any increase in the yield of oats.

EFFECT OF CROWN RUST INFECTION ON WATER REQUIREMENT AND YIELD OF GREENHOUSE-GROWN OATS

The data obtained from the field experiment conducted in 1928 and from numerous other field observations have shown that oats infected with crown rust yield less than rust-free oats and that the subsequent reduction in bushel weight and yield is positively correlated with the earliness and severity of infection. The latter is measured both by degree and type of infection. A highly resistant or nearly immune plant may be as extensively infected as a completely susceptible plant, although this will not ordinarily be the case under field conditions. It is important, however, to know what relation, if any, there is between type of infection and reduction in yield. Since soil moisture and crown rust infection, alone or together, are often limiting factors in successful oat production, it seemed desirable to determine the interrelations of soil moisture, water requirement, rust infection, and yield. To facilitate the control and measurement of these factors, 2 sets of experiments were conducted, 1 in 1930 and 1 in 1933. Substantially the same methods were employed in both cases, except that in 1933 some plants were grown with and some without adequate soil moisture.

EXPERIMENTS IN 1930

MATERIAL AND METHODS

In the experiments conducted in the greenhouse in 1930 pure-line selections of Victoria (C. I. 2401) and Markton (C. I. 2053) were used. Victoria is immune or nearly immune from or highly resistant to each of the 33 physiologic forms of *Puccinia coronata avenae* studied by the writer (7), while Markton is moderately or completely susceptible. One hundred and five 1-gallon glazed jars, each containing 4 kg of a 3-to-1 mixture of greenhouse compost and sand of uniform fertility and moisture, were used for this experiment. In order to add water to the soil directly and conveniently, an inverted 2½-inch clay flower-pot resting on a glass plate 3 inches square was placed 2 inches from the bottom of each jar and the soil was then added. A glass tube extended from within the clay pot to 3 inches above the top of the jar. Six primary grains of Victoria oats were sown in each of 50 jars on January 22; similarly, 50 jars were sown with Markton, leaving 5 jars without seed. One kilogram of clean white sand was placed on top of the soil in each jar to reduce surface evaporation. The moisture content of the soil and sand when placed in the jars was 13.95 and 3.25 percent, respectively. After the seed was sown, the soil moisture in each jar was raised to 60 percent of its water-holding capacity, or 24 percent of the dry weight, and held within a range of 55 to 65 percent of saturation by adding water at necessary intervals. The evaporation through the sand cover was estimated from the loss in weight

of the 5 jars without seed, which were kept at the optimum soil moisture. The temperature was maintained within the range 65° to 85° F.

After emergence, the number of plants was reduced to 4 per jar. When the plants were in the seedling (four-leaf) stage, 10 jars of each set were dusted with urediospores of physiologic form 1 of crown rust. A like number of each set were dusted in the early boot, early anthesis, and early dough stages, respectively, and each of the four lots was reinoculated every 2 weeks. Inoculation was accomplished by placing the plants in a large glass incubation chamber, spraying them with tap water, and dusting them with rust spores. They were allowed to remain in the chamber for 24 hours before removal to the greenhouse bench.

The dry weight of the grain, straw,⁵ and roots was obtained for all the plants used in the experiments conducted in 1930 and 1933. In each experiment, the plants when mature were harvested simultaneously. Of those that developed no panicles because of rust, some, though dying, were still partly green when harvested. The roots were harvested by washing away the soil with a light stream of water. The loss of parts of the smaller roots was probably proportional for each series and insufficient to affect the significance of the differences in yield of roots between different series. The grain was carefully hand-threshed.

RESULTS

The susceptible Markton plants initially inoculated in the seedling stage failed to head and produced 35.2 and 4.4 percent as much straw and roots, respectively, as the rust-free plants of the check (tables 3 and 4). The water requirement⁶ of these plants was almost four times that of the nonrusted plants. Although the plants were first inoculated in the four-leaf stage, they were not entirely dead when harvested. The 2-week interval between inoculations permitted a certain degree of recuperation, especially when the plants were young. Nearly as striking was the reduction in yield of the plants initially inoculated in the early boot stage. The development of the panicles was almost completely arrested when the resulting infection appeared, and the few that did emerge were barren. At the time of anthesis the grain and roots were still susceptible to considerable retardation, while the rest of the plant was apparently much less affected when inoculated at this time. The water requirement of the plants initially inoculated at this stage of development was 42.3 percent greater than that of rust-free plants. There was no significant effect on either yield or water requirement of those plants initially inoculated in the dough stage.

⁵ Straw includes everything except grain and roots.

⁶ The term "water requirement" as used herein refers to the ratio of the weight of water absorbed by a plant during its entire growth period to the weight of dry matter produced, including that of the roots.

TABLE 3.—Mean yield and water requirement, per jar of four plants, of pure-line selections of Markton and Victoria oats grown in the greenhouse and infected with physiologic form 1 of *Puccinia coronata avenae* at different stages of development, in 1930

MARKTON (SUSCEPTIBLE)

Stage at initial infection	Average weight				Average water used	Average water requirement
	Grain	Straw	Roots	Total		
	Grams	Grams	Grams	Grams	Kilograms	
Seedling	0.0	24.7±0.3	0.7±0.1	25.4±0.3	21.2	833.9±12.5
Boot	0	35.1±.2	2.0±.1	37.1±.2	22.6	610.2±2.7
Anthesis	14.1±0.5	61.8±.5	4.7±.6	80.6±.6	24.5	303.6±2.2
Dough	25.1±.3	71.0±.5	14.8±.3	110.9±.9	24.0	222.1±2.5
Check	25.8±.1	70.2±.3	16.0±.2	112.0±.3	23.9	213.4±3.1

VICTORIA (RESISTANT)

Seedling	12.6±0.2	54.5±0.5	9.2±0.2	76.3±0.7	23.9	313.0±2.2
Boot	16.6±.4	57.9±.4	11.3±.1	85.8±.7	23.8	277.3±2.2
Anthesis	19.6±.4	61.3±.5	16.3±.5	97.2±1.1	23.9	245.6±2.4
Dough	23.6±.2	65.6±.4	20.1±.5	109.3±.9	24.2	221.4±1.8
Check	24.0±.2	63.9±.3	20.3±.4	108.2±.5	24.2	223.7±1.1

TABLE 4.—Mean yield and water requirement of Markton and Victoria oats infected with *Puccinia coronata avenae* at different stages of development, in 1930

MARKTON (SUSCEPTIBLE)

Stage at initial infection	Yield ¹ of —				Water requirement ¹
	Grain	Straw	Roots	Total	
	Percent	Percent	Percent	Percent	Percent
Seedling	0.0	35.2	4.4	22.7	390.8
Boot	0	50.0	12.5	33.1	285.9
Anthesis	54.7	88.0	29.4	72.0	142.3
Dough	97.3	101.1	92.5	99.0	104.1
Check	100.0	100.0	100.0	100.0	100.0

VICTORIA (RESISTANT)

Seedling	52.5	85.3	45.3	70.5	139.9
Boot	69.2	90.6	55.7	79.3	124.0
Anthesis	81.7	95.9	80.3	89.8	109.8
Dough	98.3	102.7	99.0	101.0	99.0
Check	100.0	100.0	100.0	100.0	100.0

¹ Percentage of that of rust-free check.

As would be expected, the yield and water requirement of the resistant Victoria selection were not affected nearly so much by crown rust infection as were those of the susceptible Markton selection. Victoria plants initially infected in the seedling stage were affected to about the same degree as Markton plants initially infected during anthesis. The value and significance of the differences between the mean yields and water requirements of the different series for both selections are shown in table 5.⁷

⁷ The value of *t* and its significance were calculated and determined by the method and tables given by Fisher (1) and Snedecor (2). The value of *t* represents the ratio of the mean difference to the standard deviation of the mean difference. As the value of *t* becomes larger the significance of the mean difference becomes greater. With 18 degrees of freedom, the least value of *t* that can be considered significant is 2.1 and the least value that can be considered highly significant is 2.9.

TABLE 5.—Significance of differences in mean yield and water requirement of Markton and Victoria oats infected with *Puccinia coronata avenae* at different stages of development, in 1930

[Values in boldface type are highly significant (indicating a level of significance beyond Fisher's 1-percent point, odds 99 to 1 or greater)]

MARKTON (SUSCEPTIBLE)

Stages at initial infection	Mean difference and <i>t</i> value									
	Grain		Straw		Roots		Total weight		Water requirement	
	MD	<i>t</i>	MD	<i>t</i>	MD	<i>t</i>	MD	<i>t</i>	MD	<i>t</i>
Check seedling	25.8	166.7	45.5	82.1	15.3	49.8	86.6	137.6	320.5	36.0
Check boot	25.8	166.7	35.1	78.5	11.0	44.6	74.9	139.0	306.8	72.6
Check anthesis	11.7	46.5	8.4	11.3	11.3	46.4	31.1	33.7	90.2	17.8
Check dough	7	1.7	— 8	9	1.2	2.0	1	9	8.7	1.6
Boot seedling	0	0	10.4	20.0	1.3	11.9	11.7	21.5	223.7	13.1
Anthesis boot	14.1	76.7	26.7	37.0	2.7	22.5	43.5	49.4	306.6	67.0
Dough anthesis	11.0	33.1	9.2	8.4	10.1	25.5	30.3	21.1	81.5	18.7

VICTORIA (RESISTANT)

Check seedling	11.4	28.5	9.4	11.0	11.1	17.3	31.9	29.4	89.3	27.9
Check boot	7.4	12.5	6.0	8.5	9.0	14.8	22.4	20.3	53.6	16.4
Check anthesis	4.4	7.5	2.6	3.4	4.0	4.8	11.0	7.2	21.9	6.3
Check dough	4.4	9	— 1.7	2.4	2	3	1	8	2.3	— 8
Boot seedling	4.0	6.6	3.4	3.7	2.1	6.5	9.5	7.8	35.7	8.7
Anthesis boot	3.0	3.8	3.4	4.1	5.0	8.0	11.4	6.8	31.7	7.3
Dough anthesis	4.0	6.6	4.3	5.1	3.8	4.4	12.1	6.7	21.2	6.1

Markton is completely susceptible to crown rust physiologic form 1, while Victoria is highly resistant. The uredia on Markton were large, with no necrosis and little or no chlorosis immediately surrounding them. In the case of Victoria the uredia were comparatively few, very small, and always surrounded by necrotic areas; also, there were numerous necrotic areas that failed to produce uredia. The percentage of actual infection on the two varieties was approximately equal throughout the investigation and was regularly 90 to 100 percent following each inoculation. The effect of the rust on yield and water requirement was much greater for Markton (figs. 1 and 2). Plants of both varieties initially inoculated in the seedling, early boot, and first anthesis stages of development showed a highly significant reduction in yield of all plant parts and a consequent increase in water requirement as compared with those of the rust-free plants. In each variety there were also highly significant differences between the effects of the rust on these different series. Infection resulting from inoculation made in the dough stage evidently was too late to affect significantly the yield and hence the water requirement of either variety.

In the greenhouse, where the soil moisture was held nearly constant and optimum, crown rust infection retarded both heading and ripening (fig. 3). Markton plants initially inoculated in the seedling and early boot stages failed to head at all, while in the remaining series all of the plants headed at the same time. Those plants initially inoculated in early anthesis matured about 4 days later than the rust-free plants. Victoria plants, inoculated in the seedling, early boot, early anthesis, and dough stage, were in full head on May 22, 17, 12, and 12, respectively. The dates of ripening were correspondingly affected.

The reduced yield of grain of Markton resulted from complete absence of kernels on those plants initially inoculated in the seedling and early boot stages and fewer and lighter kernels for those first inoculated in early anthesis (fig 4). Rust infection during anthesis caused much floral sterility and reduction in size of grains. The percentage of hulls also was definitely affected. The grain harvested from those plants first infected in this stage of development contained 31.4 percent of hulls, while that from the rust-free plants contained only 20.6 percent. Crown rust infection of Victoria, even when started in the seedling stage, seemed to cause little sterility. Most of the reduction in yield of grain was apparently due to smaller and lighter kernels (fig. 5). The effect of rust on the percentage of hulls

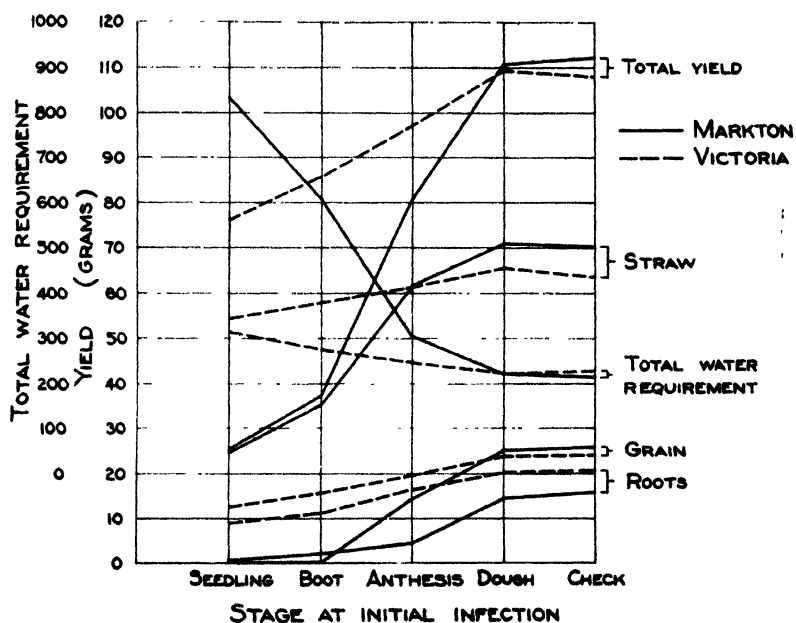


FIGURE 1.—Yield and water requirement of a susceptible selection of Markton oats and a resistant selection of Victoria oats initially infected with form 1 of crown rust in seedling, boot, anthesis, and dough stage in 1930.

in the grain harvested from the different series of Markton and Victoria is shown in table 6.

TABLE 6. - Effect of rust on percentage of hulls in grain from Markton and Victoria oats, 1930

Stage at initial infection	Markton	Victoria
Seedling	Percent (1)	Percent 29.2
Boot...	(1)	27.9
Anthesis	31.4	26.1
Check...	20.6	25.0

¹No grain.

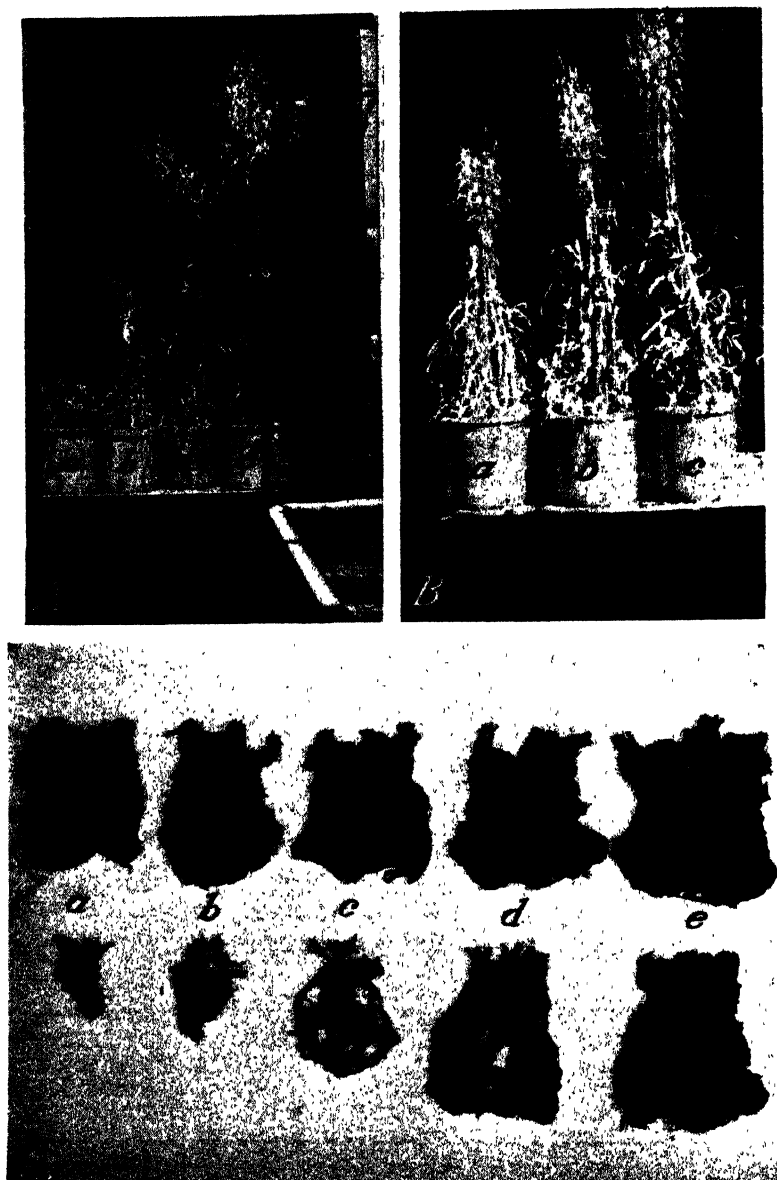


FIGURE 2.—Effect of crown rust infection on yield of a susceptible selection of Markton oats and a resistant selection of Victoria oats: *A*, Markton, initially infected in (a) seedling, (b) boot, and (c) anthesis stage; (d) check. *B*, Victoria, initially infected in (a) seedling, (b) boot, and (c) anthesis stage. *C*, Roots from Victoria (upper) and Markton (lower) initially infected in (a) seedling, (b) boot, (c) anthesis, and (d) dough stage; (e) check.

The average quantity of water used weekly by the Markton and Victoria plants in the different series is shown in figures 6 and 7. The data for plants inoculated first in the dough stage are not included because in neither variety did they differ significantly from the uninoculated plants. Although the water requirement of both varieties was greatly increased as a result of rust infection, the total water consumption of the infected plants initially inoculated in the seedling and boot stages was lower than that of the rust-free plants (table 3). The retardation of growth caused by the rust and the consequent reduction in yield resulted in a lower total water consumption but in a higher ratio of water to total dry weight. The direct effect of



FIGURE 3.—Effect of crown rust infection on heading of (A) Markton oats and (B) Victoria oats, initially inoculated in (a) seedling, (b) boot, and (c) anthesis stage. Photographed May 10, 1930, at the time of the anthesis stage inoculation.

crown rust infection on transpiration is evident following the initial inoculation of the Markton plants infected first in the seedling, boot, and anthesis stages (fig. 6). Coincident with the appearance of uredia, the water loss from the initially inoculated plants in these series showed a significant increase over that from similar noninfected plants. This difference was evident until the infected tissues began to die, i. e., about 2 weeks after the appearance of uredia. A similar but less striking difference was evident in the water consumption of initially inoculated Victoria plants. Since the degree of infection on the two varieties was approximately equal, it appears that infection with many large uredia and no necrosis causes greater retardation of growth and destruction of tissue, as well as greater increase in transpiration, than does infection with a few small uredia and much necrosis.

EXPERIMENTS IN 1933

MATERIAL AND METHODS

An experiment somewhat similar to the one just described was conducted in 1933, except that 4-gallon instead of 1-gallon jars were used, and ground cork rather than sand was employed to prevent evaporation. Sixty-five jars, each containing 15 kg of greenhouse compost and sand in the ratio of 3 to 1, with an inverted 4-inch flowerpot as a water reservoir, were used. Primary grains of three pure lines of oats, selected from the varieties Markton (C. I. 2053), Victoria (C. I. 2401), a pure-line selection susceptible to form 7, and Bond (C. I. 2733), were planted, 20 grains per jar, with 20 jars for each pure line. After emergence the number of plants per jar was reduced to 15. Five



FIGURE 4.—Mechanically separated random samples of primary (larger) and secondary (smaller) grains from a susceptible selection of Markton oats: *A*, noninfected; *B*, initially infected in the anthesis stage with form 1 of crown rust in 1930.

jars were not planted. The moisture content of the soil when added to the jars was 11 percent of the dry weight. Immediately after planting, the moisture content of the soil of 12 jars of each pure line and of 3 of the jars without seed was brought up to 85 percent of its water-holding capacity, while that of the remaining 8 jars of each pure line and of the 2 jars without seed was raised to 50 percent of its water-holding capacity. The soil in these two sets was maintained at a moisture content of 80 to 90 percent and 45 to 55 percent of saturation, respectively.

Plantings were made on April 9, 11 weeks later than for the similar studies in 1930. This allowed for the longer days and higher temperatures that more nearly correspond to those of the field. No

attempt was made to maintain a constant temperature, and in June and July the temperature in the greenhouse was often 90° to 100° F. The degree of infection on the inoculated plants was held as near as

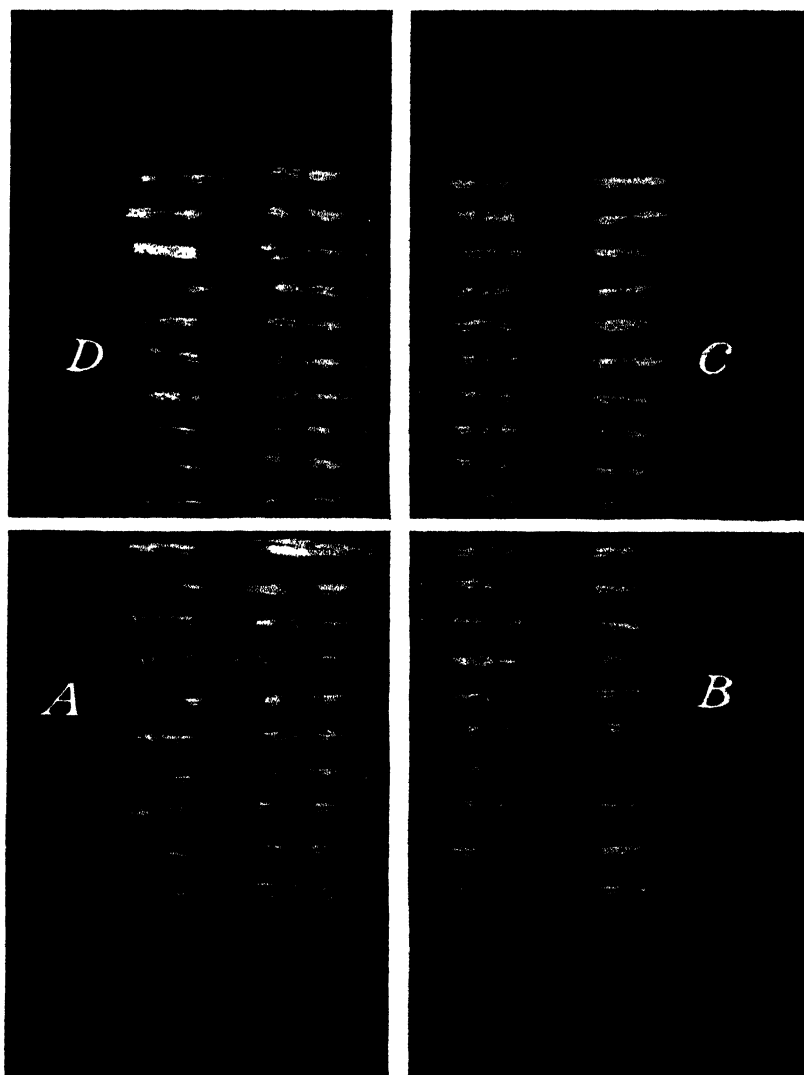


FIGURE 5. Mechanically separated random samples of primary (larger) and secondary (smaller) grains from a resistant selection of Victoria oats: A, noninfected; B-D, initially infected in (B) seedling, (C) boot, and (D) anthesis stage with form 1 in 1930.

possible to 50 percent, and that on the susceptible selections always within the range of 40 to 70 percent.

The plants in 5 jars of each variety (3 with 85 percent and 2 with 50 percent soil moisture) were initially inoculated in the seedling stage with form 7 of crown rust and reinoculated every 2 weeks until maturity. Similarly, plants of each pure line in an equal number of jars

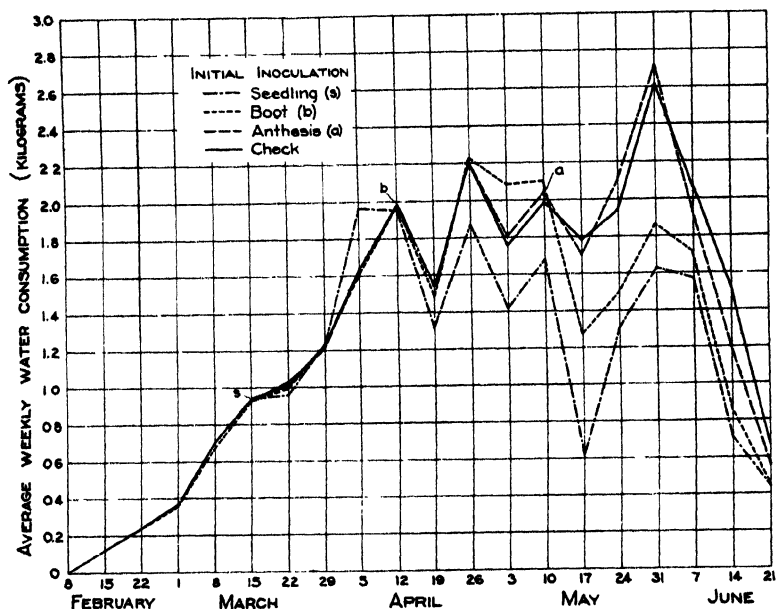


FIGURE 6.—Average weekly water consumption of a susceptible selection of Markton oats initially inoculated in seedling (s), boot (b), and anthesis (a) stage, in comparison with the noninfected check, in 1930.

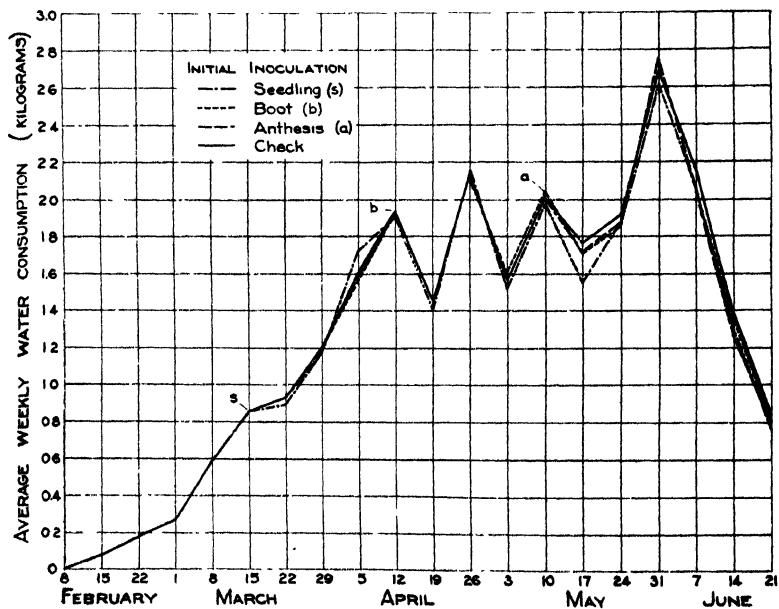


FIGURE 7.—Average weekly water consumption of a resistant selection of Victoria oats initially inoculated in the seedling (s), boot (b), and anthesis (a) stage in comparison with the noninfected check, in 1930.

were initially inoculated in the boot and early anthesis stages, while one set was retained as an uninoculated check. The plants were inoculated in the manner previously described, except that the urediospores were diluted with pure talcum powder before dusting. Initial inoculations in the dough stage were omitted in 1933 because infection initiated in this stage of development in 1930 did not affect significantly the yield or water requirement of either Markton or Victoria.

RESULTS

The yield and water requirement of Markton, Victoria, and Bond, grown at 85- and 50-percent soil moisture and first inoculated with form 7 of crown rust in the seedling, boot, and anthesis stages, are shown in tables 7 and 8. The significance of the variation in yield due to experimental error or variation within classes, effect of rust infection initiated in different stages of the development of the host, effect of soil moisture, and the interaction or change in difference from stage to stage was determined by the method of analysis of variance described by Snedecor (9).⁸ The values of the mean squares and their significance are shown in table 9.

TABLE 7.—Mean yield and water requirement, per jar of 15 plants, of pure-line selections of Markton, Victoria, and Bond oats grown in the greenhouse and infected with physiologic form 7 of *Puccinia coronata avenae* at different stages of development, in 1933

MARKTON (SUSCEPTIBLE)

Stage at initial infection	Soil moisture (percentage of saturation)	Average weight				Average water used	Average water requirement
		Grain	Straw	Roots	Total		
		Grams	Grams	Grams	Grams	Kilograms	
Seedling	85	0.5	30.8	3.1	34.4	18.1	527
	50	.5	27.7	2.7	30.8	10.6	344
Boot	85	5.7	44.6	6.3	56.6	28.0	404
	50	4.5	40.4	5.7	50.6	13.3	263
Anthesis	85	14.6	90.7	11.0	86.3	28.9	335
	50	10.2	47.0	8.7	65.9	13.0	196
Check	85	23.8	68.4	14.2	106.4	28.6	260
	50	15.7	54.5	11.1	81.3	13.5	166

VICTORIA (SUSCEPTIBLE)

Seedling	85	1.0	36.8	3.8	41.6	22.1	532
	50	1.2	30.6	2.8	34.6	13.8	399
Boot	85	5.6	45.9	8.7	60.2	27.2	452
	50	5.0	40.6	7.5	53.1	15.4	290
Anthesis	85	15.7	59.3	12.5	37.5	29.6	338
	50	11.8	46.3	9.7	67.8	15.1	223
Check	85	28.4	63.7	20.5	112.6	30.6	272
	50	19.8	50.4	16.7	86.9	16.3	188

BOND (NEARLY IMMUNE)

Seedling	85	19.6	59.4	14.4	93.4	28.2	302
	50	14.3	41.8	10.5	66.6	15.1	227
Boot	85	20.1	61.5	15.1	96.7	28.6	296
	50	15.9	45.9	13.3	75.1	16.5	220
Anthesis	85	21.8	64.5	15.8	102.1	29.3	290
	50	18.0	47.7	14.1	79.8	17.6	221
Check	85	22.8	65.6	15.8	104.2	29.7	285
	50	18.4	50.4	14.8	83.6	17.9	214

⁸ The significance of the mean squares was determined by calculating the ratio of the larger mean square to the smaller. This ratio is designated as *F* by Snedecor. As the value of *F* becomes larger the significance becomes greater, depending upon the degrees of freedom in the two mean squares. With 3 and 12 degrees of freedom the least value of *F* that can be considered highly significant is 6.0, and with 1 and 12 degrees of freedom the least value that can be considered highly significant is 9.3.

TABLE 8.—Mean yield and water requirement¹ of Markton, Victoria, and Bond Oats infected with *Puccinia coronata avenae* at different stages of development, in 1933

GRAIN						
Stage at initial infection	Markton (susceptible)		Victoria (susceptible)		Bond (nearly immune)	
	High soil moisture	Low soil moisture	High soil moisture	Low soil moisture	High soil moisture	Low soil moisture
	Percent	Percent	Percent	Percent	Percent	Percent
Seedling	2.1	3.2	3.5	6.1	86.0	77.7
Boot	23.9	28.7	19.7	25.3	88.2	86.4
Anthesis	61.3	65.0	55.3	59.6	95.6	97.8
Check	100.0	100.0	100.0	100.0	100.0	100.0

STRAW						
Seedling	45.0	50.8	57.8	60.7	90.5	82.9
Boot	65.2	71.1	72.1	80.6	93.8	91.1
Anthesis	88.7	86.2	93.1	91.9	96.8	94.6
Check	100.0	100.0	100.0	100.0	100.0	100.0

ROOTS						
Seedling	21.8	24.3	18.5	16.8	91.1	70.9
Boot	44.4	51.4	12.4	44.9	95.6	89.9
Anthesis	77.5	78.4	61.0	58.1	100.0	95.3
Check	100.0	100.0	100.0	100.0	100.0	100.0

WATER REQUIREMENT						
Seedling	195.9	207.2	195.6	212.3	106.0	106.1
Boot	183.6	158.4	166.2	154.3	103.9	102.8
Anthesis	124.5	118.1	124.3	118.6	101.8	103.3
Check	100.0	100.0	100.0	100.0	100.0	100.0

¹ Percentage of that of rust-free checkTABLE 9.—Analysis of the variation in yield and water requirement of Markton, Victoria, and Bond oats infected with physiologic form 7 of *Puccinia coronata avenae*, in 1933

[Values in boldface type are highly significant (level of significance beyond Fisher's 1-percent point, odds 99 to 1 or greater)]

MARKTON (SUSCEPTIBLE)						
Source of variation	Degrees of freedom	Mean squares				
		Grain	Straw	Roots	Total weight	Total water requirement
Experimental error	12	0.17	0.58	0.23	2.88	185.42
Between stages	3	410.52	1,064.56	96.82	3,965.79	54,926.85
Between moistures	1	61.20	369.26	12.22	940.75	129,109.80
Interaction	3	21.64	41.63	2.09	142.15	3,672.78

VICTORIA (SUSCEPTIBLE)						
Experimental error	12	0.60	1.86	0.65	5.46	211.72
Between stages	3	556.64	582.02	213.26	3,776.76	56,462.13
Between moistures	1	50.96	482.06	28.28	1,072.21	73,597.60
Interaction	3	19.21	21.76	2.14	164.89	1,291.14

BOND (NEARLY IMMUNE)						
Experimental error	12	1.76	2.03	0.85	11.19	178.89
Between stages	3	14.53	46.42	6.54	170.09	204.32
Between moistures	1	92.68	1,237.13	21.65	2,443.82	24,884.25
Interaction	3	.38	1.38	1.65	8.56	16.68

The 1933 yields were much lower than those of 1930, probably because of the excessive heat. A 50-percent infection with form 7 on Markton caused a decrease in yield and an increase in water requirement smaller than those caused by a 100-percent infection with form 1 in 1930. Crown rust infection again reduced the yield of grain more than that of any other part of the plant; the roots were the parts next most affected, and the straw, or remaining plant parts, were the least affected. Rust infection caused a greater reduction in the yield of the susceptible Markton oats when grown at 85-percent soil moisture than when grown at 50 percent (fig. 8). The

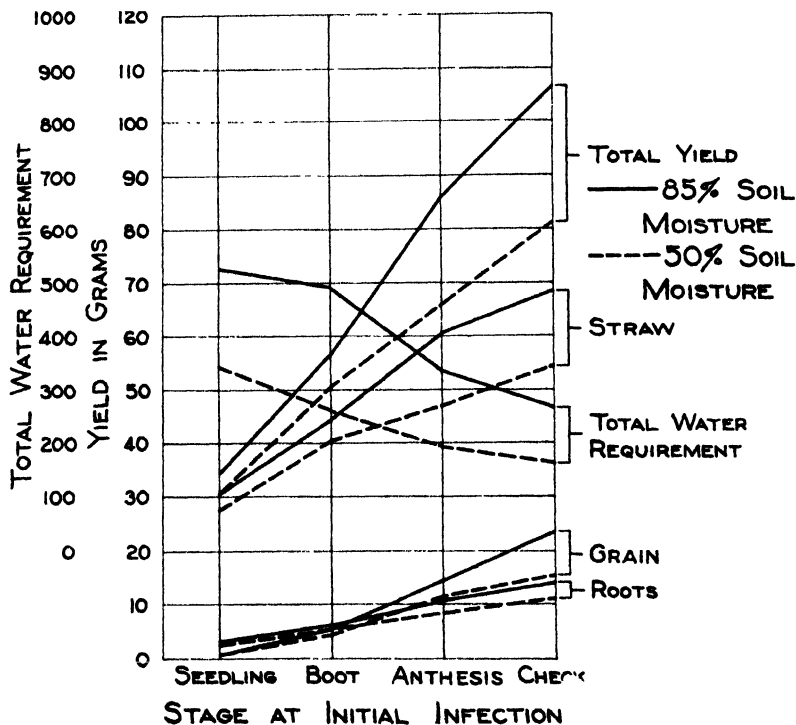


FIGURE 8. Yield and water requirement of a susceptible selection of Markton oats grown at 85- and 50-percent soil moisture and initially infected in seedling, boot, and anthesis stage with form 7 of crown rust, in 1933.

increase in total water requirement due to infection was greater at 85-percent soil moisture for those plants initially inoculated in the boot and anthesis stages but lower when inoculations were started in the seedling stage. Plants growing at a soil moisture of 50 percent regularly yielded significantly less than those growing at 85-percent soil moisture, regardless of the time of initial infection. Low soil moisture decreased the yield of grain more than that of any other plant structure, but caused an even more significant decrease in the water requirement of all plants.

The pure-line selection of Victoria used in 1933 was susceptible to form 7. This was not the same reaction as that shown by the parent variety or the pure-line selection used in 1930. The selections, how-

ever, appeared to be identical morphologically. The effect of infection with form 7 on the yield and water requirement of the susceptible pure line of Victoria (fig. 9) was very similar to the effect on Markton. The increase in total water requirement for each variety was greater at 85-percent soil moisture for those plants initially inoculated in the boot and flower stages, but lower when inoculations were started in the seedling stage. The decrease in yield due to rust infection was, with two exceptions, regularly greater at the higher soil moisture, although the differences usually were small. Both varie-

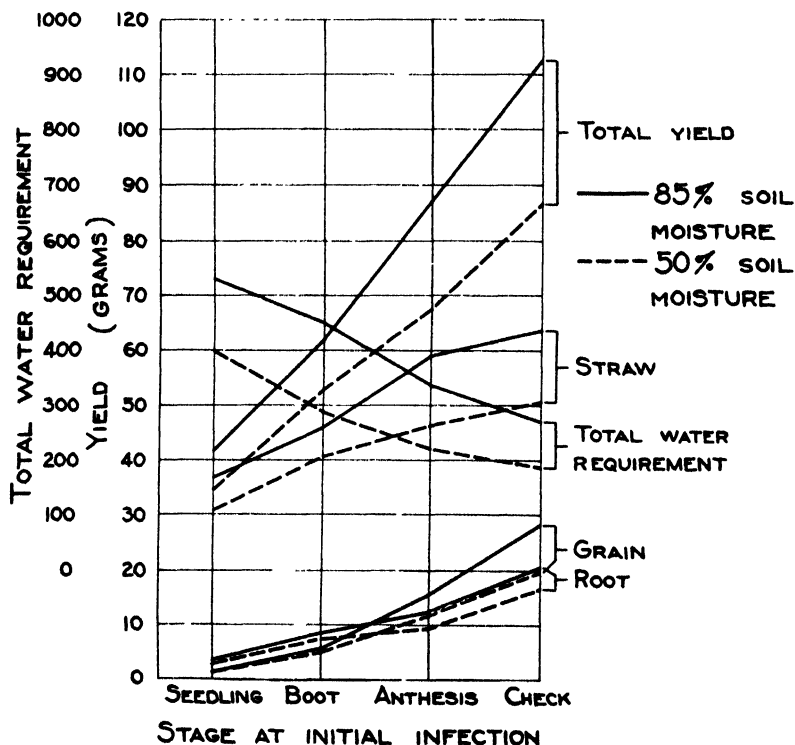


FIGURE 9. Yield and water requirement of a susceptible selection of Victoria oats grown at 85- and 50-percent soil moisture and initially infected in the seedling, boot, and anthesis stage with form 7 of crown rust, in 1933

ties, with one exception, regularly showed significantly higher yields at the higher soil moisture.

The yield and water requirement of the nearly immune pure-line selection of Bond, when infected in the various stages with form 7, is illustrated in figure 10. Although the quantity of inoculum applied was always equal to if not greater than that applied to the susceptible Markton and Victoria selections, the percentage of infection on Bond was consistently about 15 and never more than 30. As previously mentioned, uredia were entirely absent, chlorotic or slightly necrotic flecks being the only macroscopic evidence of infection. This slight and resistant type of infection, however, evidently caused a significant retardation in the development and yield of the plants.

The experiments conducted in 1933 show that infection with form 7 decreased the yield of the susceptible pure lines of Markton and Victoria to a relatively greater extent at a soil moisture of 85 percent than at a soil moisture of 50 percent. The lower soil moisture significantly reduced the yield of all plant parts of the susceptible selections, but not so much as did infection initiated in either the seedling or the boot stage of the host. Crown rust affected the yield of grain of these same selections more profoundly than did the lower soil moisture, while in the case of the Bond selection the low soil

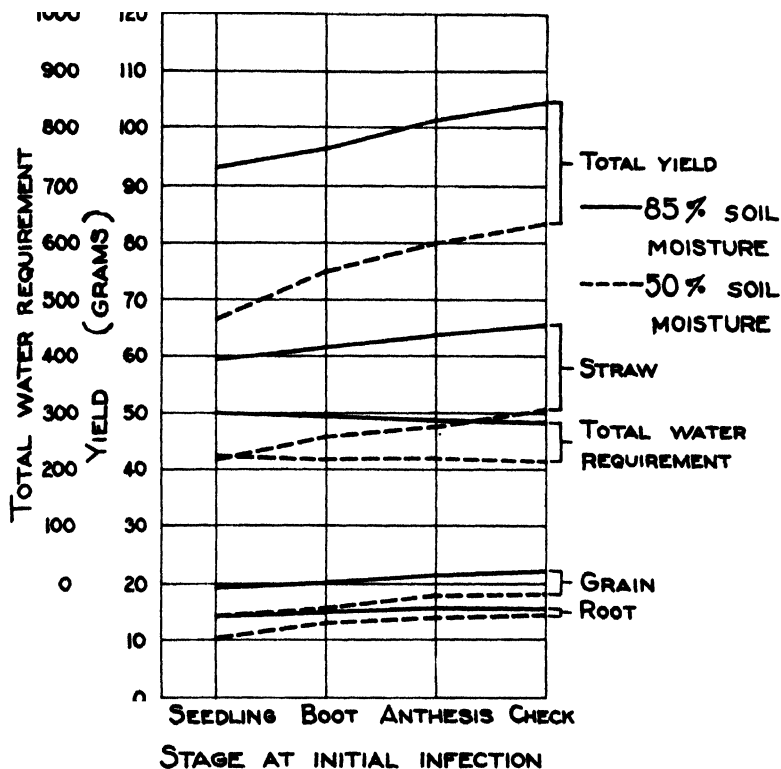


FIGURE 10. --Yield and water requirement of a nearly immune selection of Bond oats grown at 85- and 50-percent soil moisture and initially infected in the seedling, boot, and anthesis stage with form 7 of crown rust, in 1933.

moisture affected the yield more than did the rust infection, even when the latter was initiated in the seedling stage. In general, on both susceptible and resistant selections the yield of grain was most affected by rust, the yield of roots somewhat less so, and the yield of straw least. Rust infection caused a higher water requirement on both susceptible and resistant selections, but the effect was many times greater on the former; and the earlier the infection of either, the more pronounced the effect. The lower soil moisture, although it greatly decreased the yields of all series, brought about a much lower water requirement.

EFFECT OF CROWN RUST INFECTION ON THE RATIO OF ROOTS TO STRAW

Crown rust infection, as it occurred on the plants studied under greenhouse conditions in 1930 and 1933, was limited primarily to the leaf blades and leaf sheaths, with some infection frequently present on the stems and panicles. Infection was not observed on the grains, although the glumes were often slightly infected. The roots obviously were free from rust. All of the tissues included as straw were more or less infected; and, although most of the food used by the plant is manufactured or elaborated in these same tissues, the yield of straw was least affected by the rust, while the yield of rust-free grain and roots was greatly reduced.

Crown rust apparently affects the yield of grain and roots indirectly by reducing the food supply available for translocation to these parts. Long (5) found that a heavy infection of crown rust on a susceptible oat variety reduced the photosynthetic activity of the host 52 percent. Her data indicate that rust infection interfered seriously with the accumulation of reducing sugars and impaired by more than 50 percent the ability of infected tissues to elaborate carbohydrates. Any factor thus affecting the photosynthetic activity of the host would tend to decrease the ratio of the roots to the tops or straw because of the diminished translocation of food materials to the roots. Turner (10) reports that barley plants grown in the shade had a lower ratio of roots to tops than similar plants grown in sunlight. Crown rust brings about a reduction in the yield of all plant parts, but most reduction occurs in those parts most distant from the point of infection, which in this case are the parts farthest from the source of elaborated foods, as indicated in table 10, which shows the ratio of roots to straw in the experiments conducted in 1930 and 1933.

TABLE 10.--*Effect of crown-rust infection on root and straw ratios*

Stage at initial infection	Weight of roots as percentage of the weight of straw							
	1930		1933					
	Mark-ton	Vic-toria	Mark-ton		Victoria		Bond	
			High soil moisture	Low soil moisture	High soil moisture	Low soil moisture	High soil moisture	Low soil moisture
Seedling	2.8	16.9	10.1	9.7	10.3	9.2	24.2	25.1
Boot	5.7	19.5	14.1	14.1	19.0	18.5	24.6	29.0
Anthesis	7.6	28.6	18.1	18.5	21.1	21.0	24.5	29.6
Dough	20.8	30.6						
Check	22.8	31.8	20.8	20.4	32.2	33.1	24.1	29.4

The ratio of the roots and grain to straw and the ratio of roots to tops were reduced in proportion to the severity and total duration of infection. In 1930, with a 100-percent infection, the ratio of roots to straw of the susceptible Markton selection was reduced from 22.8 percent for the rust-free plants to 2.8 percent for those initially infected in the seedling stage, while the corresponding reduction for the resistant Victoria selection was from 31.8 to 16.9 percent. There is

a consistent relation between the total duration of infection and the reduction in the ratio of roots to straw for both the susceptible and resistant selections, although the reduction is much more pronounced in the susceptible selections.

The 1933 data for these crown-rust susceptible selections of Markton and Victoria were very similar to those obtained for these selections in 1930, except that the reduction in the ration of roots to straw was not so great. The relation between the duration of infection and the reduction of the ratio of roots to straw is clearly evident at both soil moistures. When infection was initiated in the seedling stage the ratio of roots to straw was smaller at the lower soil moisture, but as initial infection was delayed the ratio of roots to straw tended to become greater at the lower soil moisture. Rust had very little effect on the ratio of roots to straw of the nearly immune Bond selection, while the lower soil moisture brought about a consistent increase in the ratio of roots to straw.

The ratio of roots to tops or straw is doubtless of great importance under field conditions. Heavy epiphytotics of crown rust usually are preceded by a period of wet weather with attendant high humidity and soil moisture. These conditions, while highly favorable for rust, are also favorable for top growth, and as stated by Loomis (6), root development suffers. Then as rust appears and increases, the ratio of roots to tops is still further decreased by the effect of the fungus. This results in a topheavy plant with luxuriant foliage, heavily infected with rust, and with a dwarfed root system. Such a plant is at a great disadvantage when, as is often the case late in the season, deficient soil moisture becomes a limiting factor. The lack of a sufficient root system directly reduces the ability of the plant to obtain moisture, while, at the same time, as a result of the rust infection, its water requirement is greatly increased. The food supply is much less because of the dwarfed root system and limited water supply, while the fungus interferes directly with the photosynthetic processes of the infected plant. The pathogene, growing at the immediate source of elaborated plant food, is in a position to satisfy its own needs before translocation takes place. In consequence, the grain and roots, being farthest from these infected tissues but dependent upon them for their food supply, suffer the most serious loss.

DISCUSSION AND CONCLUSIONS

The results of these investigations in 1928, 1930, and 1933 indicate that the effect of crown rust on photosynthesis, date of ripening, total yield, and water requirement of susceptible and resistant varieties of oats varies with the degree and type of infection, the stage of growth of the host, and the duration of infection. Crown rust infection affected significantly the yield and water requirement of both susceptible and resistant varieties when it appeared at any time before the dough stage of host development. The longer the duration and the more abundant the infection the greater was the decrease in yield and the increase in water requirement. Susceptible varieties that become heavily infected early and so remain until maturity may yield no seed at all, while similar infection initiated during anthesis may cause a 50-percent reduction in the yield of grain. The yield and water requirement of a resistant variety are much less affected. An

infection with a few small uredia and much necrosis on the resistant Victoria selection in 1930 reduced the yield of grain approximately one-half as much as did an equal amount of infection with many large uredia and no necrosis on Markton. A resistant variety showing only chlorotic or necrotic flecks or a few small uredia in necrotic areas will apparently be damaged much less than a susceptible variety with an equal amount of infection in which uredia are abundant but with no surrounding necrosis and little chlorosis. For a given area chlorotic infection apparently causes less damage to the host than does a necrotic one, while an infection characterized by abundant uredia unaccompanied by any surrounding necrosis and with little chlorosis causes much more damage than either of the former. When the percentages of infection are equal, the effect of all infection types on the amount of functioning leaf area is probably about the same, but their effect on the metabolism of the host is evidently very different.

Under field conditions the amount of infection on a resistant or nearly immune variety is automatically restricted because of the partial or complete elimination of the source of inoculum for secondary and later infections. As the acreage of a resistant variety is increased the amount of inoculum available for secondary and later infections becomes less, while with a susceptible variety the amount of inoculum and the resulting infection become greatly increased. This particular advantage of a resistant variety may be easily overlooked in nursery tests where resistant and susceptible sorts are grown near each other. There resistant varieties usually are subject to severe infection from neighboring susceptible varieties and their yield may be reduced to the point where it does not surpass that of the susceptible ones, while under field conditions the resistant varieties might be far superior.

Reduced yield of grain was expressed in terms of fewer and lighter kernels. Infection initiated on susceptible selections in or before the boot stage caused partial or complete absence of kernels, depending upon the amount of infection; while infection initiated during anthesis affected mainly the size of the kernels. On resistant selections, rust infection did not appear to cause much sterility but chiefly affected the weight per kernel. The decrease in yield of grain of susceptible selections, due to rust infection, was slightly greater at the higher soil moisture. A combination of heavy rust infection and low soil moisture apparently will cause a much greater loss in yield than either one alone, even though the percentage of loss caused by the rust be slightly greater at the higher soil moisture.

Crown rust infection increased the water requirement of both susceptible and resistant plants in about the same manner and degree that it decreased the yield of grain. Lowering the soil moisture, on the other hand, decreased the water requirement. The decrease in water requirement of the susceptible selections, due to the lower soil moisture, was not sufficient to compensate for the increase caused by rust infection initiated in the seedling or boot stages but was more than equal to the increase due to infection initiated during anthesis. In the case of the resistant selection the lower soil moisture reduced the water requirement 25 percent, while rust infection initiated in anthesis increased it only 6 percent.

Rust infection probably affects the water requirement directly by mechanical injury and indirectly by its effect on the metabolism of the host. There was a perceptible increase in the water consump-

tion of initially infected plants about the time the uredia ruptured the epidermis. These infected plants continued to use more water than similar rust-free plants for a period of about 2 weeks, when the infected tissues began to die. The increased water requirement of the infected plants was mostly due to the reduction in yield, but since the retardation of growth was caused by rust, this increased water requirement and lower total consumption of water was indirectly a result of rust infection.

The data presented in this paper are of interest in that they permit of a more accurate estimation of the amount of damage that may result from a moderate or heavy infection of crown rust under field conditions. There is probably a tendency to underestimate the damage caused by crown rust because the parts of the plant actually infected suffer less than the grain and roots, which are not infected. The fact that oat plants heavily infected with crown rust may, under favorable conditions, retain a rather luxuriant foliage often causes the layman to believe that little damage is done to the plant, when, in reality, the yield of grain is greatly reduced by the rust. These data also emphasize the importance of considering the degree and duration of infection when evaluating the damage likely to occur as a result of rust infection.

Although it is evident that most of the loss caused by crown rust may be prevented by dusting with sulphur at proper intervals, the most desirable method of combating the disease is obviously that of developing rust-resistant varieties. The value of any high-yielding selections or varieties endowed with Victoria's resistance to form 1 should not be overlooked, while near immunity, such as that exhibited by the Bond selection, affords virtually complete protection from crown rust.

SUMMARY

Iomine oats growing under field conditions in 1928 and showing a 100-percent infection artificially initiated on June 9 produced 44.2 percent as much grain as sulphur-dusted oats of the same variety with 11.7 percent infection. Oats of the same variety with 24-percent infection yielded 74.1 percent as much grain as nearly rust-free ones. There was evident a strong positive correlation between early initial infection and reduction in yield and weight per bushel.

Early infection retarded the date of heading of Iomine plants, although these same plants showed premature ripening before the later heading, less rusted plants. The premature ripening of the heavily rusted plants was apparently due to their greater susceptibility to injury from the excessively hot dry weather of early July.

Two pure lines of oats, selected from Markton and Victoria, the former completely susceptible and the latter highly resistant, were grown to maturity in the greenhouse in 1930 and subjected to a 90- to 100-percent infection of form 1 of crown rust. A study was made of the relation of the length of the infection period to yield and water requirement.

Crown rust infection initiated on plants of both selections in the seedling, boot, or anthesis stage of growth resulted in a significant reduction in yield and increase in water requirement. Infection started in the dough stage of growth had no significant effect on either selection.

The reduction in yield and increase in water requirement were positively correlated with the length of the infection period.

Infection initiated in the seedling and boot stages completely checked the production of grain on the susceptible selection, while the yield of the resistant one was 52.5 and 69.2 percent, respectively, as much as that of the rust-free plants.

The reduction in grain yield was due to the production of fewer and lighter kernels. Rust infection increased the percentage of hulls in the grain.

Except where the yield of grain was entirely inhibited, the yield of roots was reduced most and that of the straw least.

Susceptible plants first infected in the seedling stage used 290.8 percent more water per unit of dry weight than rust-free plants, while resistant plants used only 39.9 percent more.

Three pure lines of oats, selected from Markton, Victoria, and Bond, the first two completely susceptible and the last nearly immune, were grown to maturity at two soil moistures in 1933. The susceptible plants were subjected to a 40- to 70-percent infection with form 7 of crown rust, while the amount of infection on the nearly immune plants was 15 to 30 percent.

The lighter infection in 1933 reduced the yield and increased the water requirement of the susceptible selections in a manner similar to but less than the heavy infection in 1930.

Rust infection tended to have a greater effect on the yield and water requirement of the susceptible selections at the higher soil moisture, while on a nearly immune Bond selection the effect was greater at the lower soil moisture.

The lower moisture significantly reduced the yield of all plant parts. With the susceptible selections this effect was less than that caused by infection initiated in the seedling or boot stage, but greater when infection was initiated in the anthesis stage, while in the case of the resistant Bond selection the lower soil moisture regularly affected the yield more than did the rust infection. Lowering the soil moisture also decreased the water requirement of all plants, both rusted and rust-free.

The ratio of roots to tops was greatly decreased by rust infection, the decrease being in proportion to the duration and severity of infection.

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FACTORS AFFECTING THE ABSORPTION OF SELENIUM FROM SOILS BY PLANTS¹

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INTRODUCTION

The cause of a serious disease of livestock in certain restricted areas of the Great Plains has recently been traced by the United States Department of Agriculture, in cooperation with the agricultural experiment stations of certain of the affected States, to ingestion of selenium contained in the vegetation (3).³ That selenium is absorbed readily by plants which thereupon become toxic to animals has been established experimentally (10). Some of the factors affecting this absorption are reported in the present paper, which supplements an earlier discussion (7) of the toxicity of selenium to plants.

METHODS⁴

The plants, mostly wheat (Hard Federation), were grown in soil to which selenium had been added in the form of sodium selenate ($\text{Na}_2\text{SeO}_4 \cdot 10\text{H}_2\text{O}$). Solutions containing the predetermined quantities of selenium, expressed as parts per million by weight of the soil, were added with thorough mixing to insure uniform distribution.

Estimates of injury to the plants (indicated by number of plus signs in the tables) were based on the occurrence and extent of the snow-white chlorosis and stunting characteristic of wheat plants damaged by sodium selenate (fig. 1). The selenium taken up by the plants, exclusive of the roots, was determined⁴ quantitatively by the distillation method (11) and expressed as parts per million of the air-dry weight of the material. Colorimetric estimations⁴ were in agreement with the results obtained by the distillation method.

RESULTS

COMPARATIVE SELENIUM ABSORPTION BY DIFFERENT CROPS

A number of different crops were grown in greenhouse benches at the Arlington Experiment Farm, Rosslyn, Va., in the local Keyport clay loam into which sodium selenate was mixed at a rate of 5 parts per million of selenium. The average temperature of the greenhouse was near 20° C. for the first 2 months, at the end of which period some of each lot of plants (except sorgo and bromegrass, which were cut a month later) were cut and analyzed (table 1). This temperature was too low to permit good growth of the high-temperature plants, millet, sorgo, and corn. Of the 17 crops, only sorgo showed specific symptoms of selenium injury, i. e., snow-white chlorosis, often tinged with pink.

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² The writer is indebted to A. G. Johnson, of the Division of Cereal Crops and Diseases, and to H. G. Byers, M. J. Horn, T. D. Rice, and W. O. Robinson, of the Bureau of Chemistry and Soils, for helpful cooperation.

³ Reference is made by number (italic) to Literature Cited, p. 426.

⁴ The quantitative selenium determinations were made by A. Van Kleeck, under the direction of H. G. Byers, of the Bureau of Chemistry and Soils. The colorimetric estimations were made by M. J. Horn with his modification of the Schmidt method (6). Analyses of the soils for sulphur were made by J. B. Martin under the direction of E. C. Shorey of the Bureau of Plant Industry.

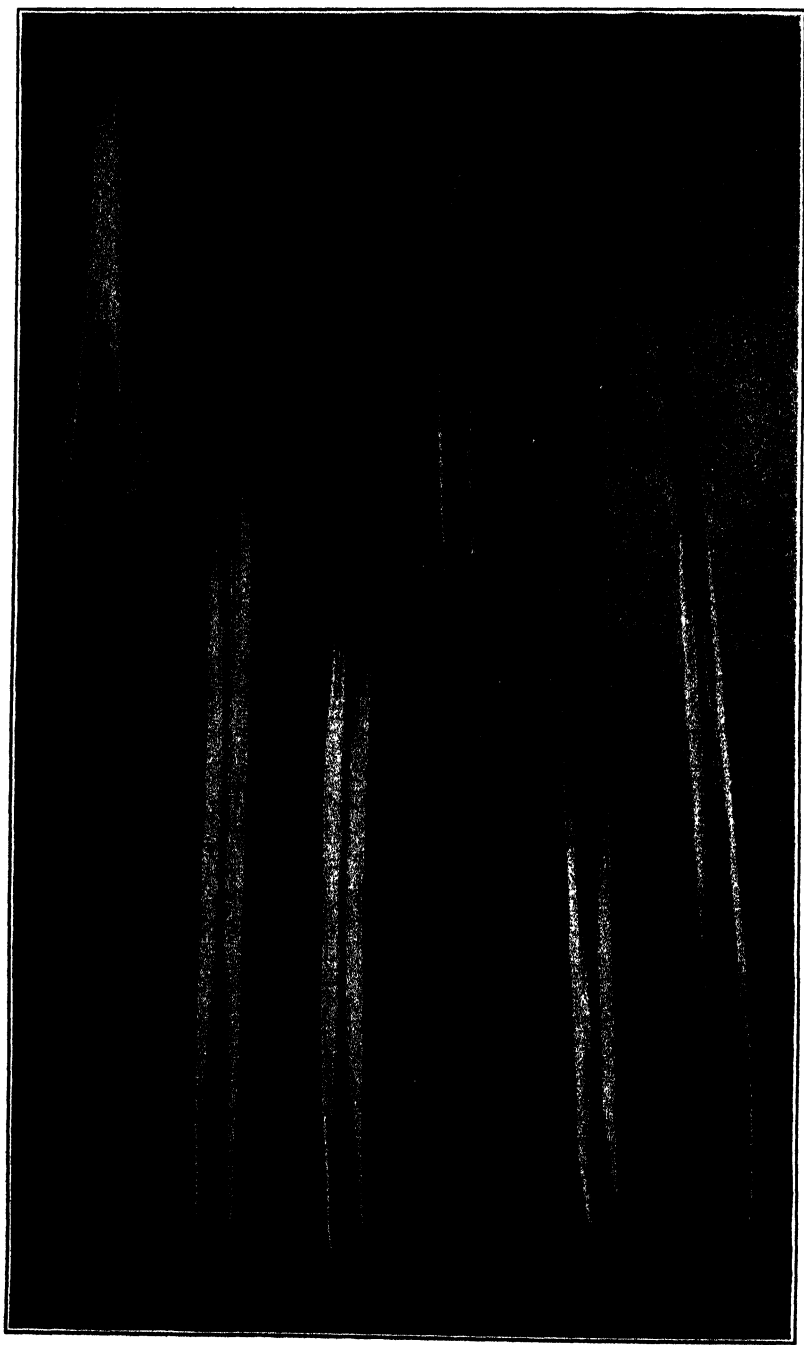


FIGURE 1.—Typical selenium chlorosis on wheat leaf

TABLE 1.—*Comparative absorption of selenium by young plants of different crops grown in the greenhouse with 5 parts per million of selenium as sodium selenate added to the soil (Keyport clay loam)*

Crop	Condition	Selenium in plants	Crop	Condition	Selenium in plants
Cruciferae:		<i>P p m.</i>	Gramineae:		<i>P. p m.</i>
Mustard	Normal	1,240	German millet	Growth slow	590
Broccoli	do	1,180	Oats	Normal	535
Compositae:			Wheat	do	470
Sunflower	Leaves spotted	790	Barley	do	450
Linaceae:			Proso millet	Growth slow	295
Flax	Normal	685	Corn	do	275
Leguminosae:			Crested wheatgrass	Normal	255
Sweetclover	do	645	Bromegrass	do	200
Alfalfa	do	560	Sorgo	Growth slow, leaves chlorotic and pink	130
Pea	do	560			
Chenopodiaceae					
Spinach	do	315			

The data in table 1 show the greatest accumulation of selenium in the two representatives of the Cruciferae—mustard and broccoli. One of the outstanding characteristics of plants of this family is their capacity for absorbing large quantities of sulphur from the soil for the synthesis of sulphur-containing compounds, such as the glucoside sinigrin, from which mustard oil is derived. If the previously suggested explanation (7) of sulphur-selenium antagonism is correct (a theory based on the assumption of substitution of selenium for sulphur in some synthesized compound in the plant) it would seem likely that the greater accumulation of selenium in the Cruciferae is to be accounted for by their capacity for high sulphur absorption. On this assumption the legumes that absorb sulphur in large quantities by virtue of their high protein content might also be expected to accumulate selenium readily, whereas the Gramineae with a low sulphur requirement should absorb less. The data show that the three representatives of the Leguminosae were relatively high in selenium, exceeding in amount that found in any of the Gramineae except German millet, but containing less than flax and sunflower.

COMPARATIVE SELENIUM ABSORPTION FROM KEYPORT CLAY LOAM AND PIERRE CLAY

Sodium selenate was much more toxic to wheat growing in Pierre clay than in Keyport clay loam. In the latter soil about 15 p. p. m. of selenium was required for distinct chlorosis and over 30 p. p. m. for fatal injury (fig. 2, A), whereas in the former the addition of only 3 p. p. m. produced definite traces of chlorosis and 10 p. p. m. was often fatal (fig. 2, B).

Comparative analyses of the leaves of plants grown subsequently in these same pots of soil indicated that the greater toxicity of selenium in the Pierre clay was associated with greater availability to the plant, more selenium being taken up than from the same concentrations in the Keyport clay loam. Thus the data in table 2 show that more was absorbed from 5 p. p. m. added to the former soil than from 10 p. p. m. in the latter, and the amount taken up from 10 p. p. m. in the former soil was of the same order of magnitude as that absorbed from 30 p. p. m. in the latter. The Pierre clay was an alkaline black gumbo soil, containing about 12 percent calcium carbonate, brought

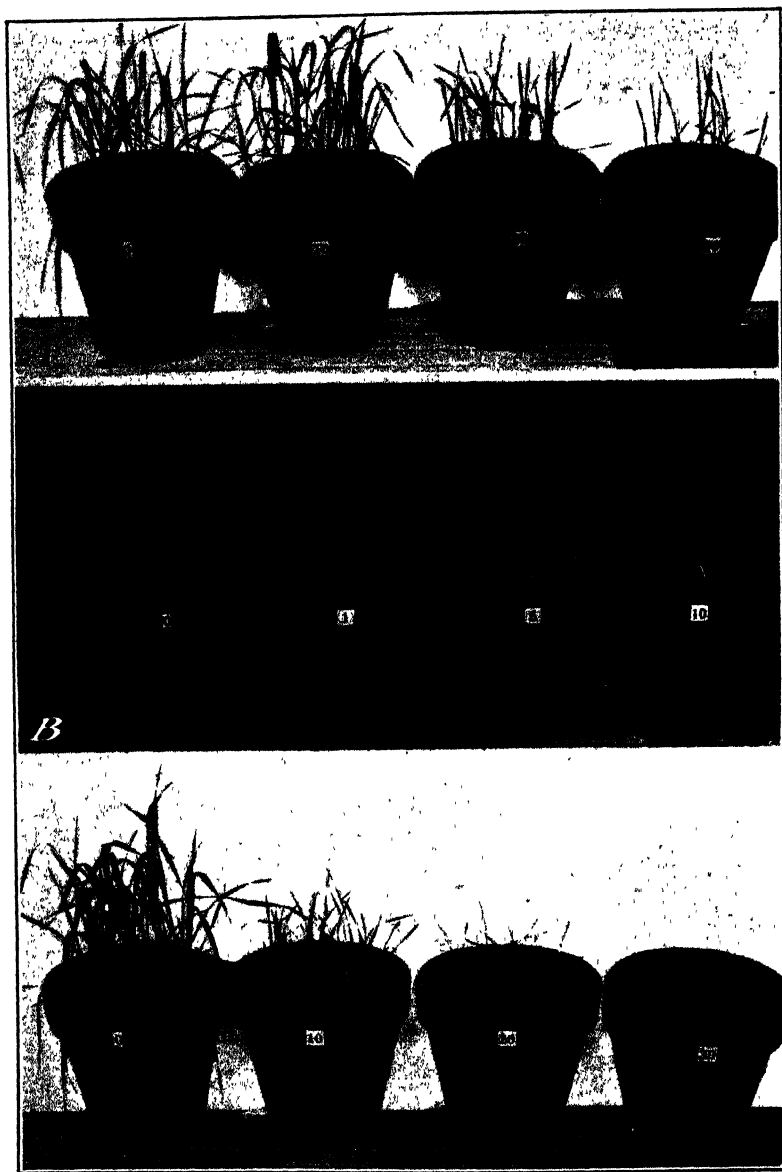


FIGURE 2—Toxicity of sodium selenate to wheat plants 5 weeks old in different soils. Numbers on pots show parts per million of selenium: *A*, Plants in Keyport clay loam, not visibly affected, by 10 parts per million of selenium, chlorotic and stunted with 20 and 30 parts per million; *B*, plants in Pierre clay normal with 1 part per million, chlorotic and stunted with 5 parts per million, dead with 10 parts per million; *C*, plants in greenhouse potting soil (clay loam) to which 20 percent of quartz sand was added, chlorotic and stunted with 10 and 20 parts per million of selenium and dead with 30 parts per million.

from a naturally seleniferous area in Gregory County, S. Dak., for the experiments. It had a trace of naturally occurring selenium, but the quantity (less than 1 p. p. m.) was so small as to produce but 15 p. p. m. in the control plants. However, for strict comparisons of the availability of the added selenate this amount should be subtracted from the total found in the plants in the Pierre clay. Thus 945 and 1,335 p. p. m. selenium were absorbed from 10 and 20 p. p. m., respectively, in this soil as compared with 380 and 530 from the corresponding additions of selenate to the clay loam.

TABLE 2.—*Absorption of selenium by wheat from sodium selenate added to Keyport clay loam and Pierre clay*

Selenium added to soil (p. p. m.)	Keyport clay loam			Pierre clay		
	Condition of plants	Degree of chlorosis	Selenium in plants	Condition of plants	Degree of chlorosis	Selenium in plants
			P p. m.			P p. m.
0	Normal	0	Trace	Normal	0	15
1				do.	0	325
3				Almost normal	+	330
5				Stunted	++	450
10	Normal	0	380	Severely stunted	+++	960
20	Stunted	++	530	Withered, almost dead	++++	1,350
30	Severely stunted	+++	1,000			
35	do	+++	1,120			

¹ The distillation method of analysis indicated the presence of a trace of selenium in these plants, but none was detected by the codeine-sulphate method

The differences in the amounts of selenium absorbed from the two soils were not due to differences in their sulphur content (7), for analyses showed but 0.003 percent of water-soluble sulphur in each. Total sulphur was 0.03 percent in the Pierre clay and 0.06 percent in the Keyport clay loam. Nor was hydrogen-ion concentration the determining factor, for increasing the alkalinity of the slightly acid Keyport clay loam (pH 6.7) to that of the Pierre clay (pH 8.1) by adding calcium carbonate did not increase the toxicity of the sodium selenate. In fact, the calcium carbonate application (1 part in 500 parts of soil) improved the condition of the plants. It has been suggested ⁵ that the difference might be due to the difference in composition of the soil colloids. The high molecular ratio of silica to the sum of the alumina and iron in the colloids of the Pierre clay, 3.5, as compared with that of the Keyport clay loam, 1.9, would result in more insoluble combination and decreased availability of the selenium in the salt added to the soil with the lower ratio (12).

SELENIUM ABSORPTION AS A FUNCTION OF THE CONCENTRATION OF AVAILABLE SULPHUR IN THE SOIL

As previously reported (6), the detection by J. E. McMurtrey, Jr., of the Bureau of Plant Industry, of a resemblance between certain aspects of selenium chlorosis and the chlorosis resulting from sulphur deficiency led to the discovery that the toxicity of sodium selenate to wheat plants is conditioned by the quantity of available sulphur or sulphates (7). The comparative amounts of sulphur required

⁵ This suggestion was made by H. G. Byers, in whose division of the Bureau of Chemistry and Soils the colloid ratios were determined.

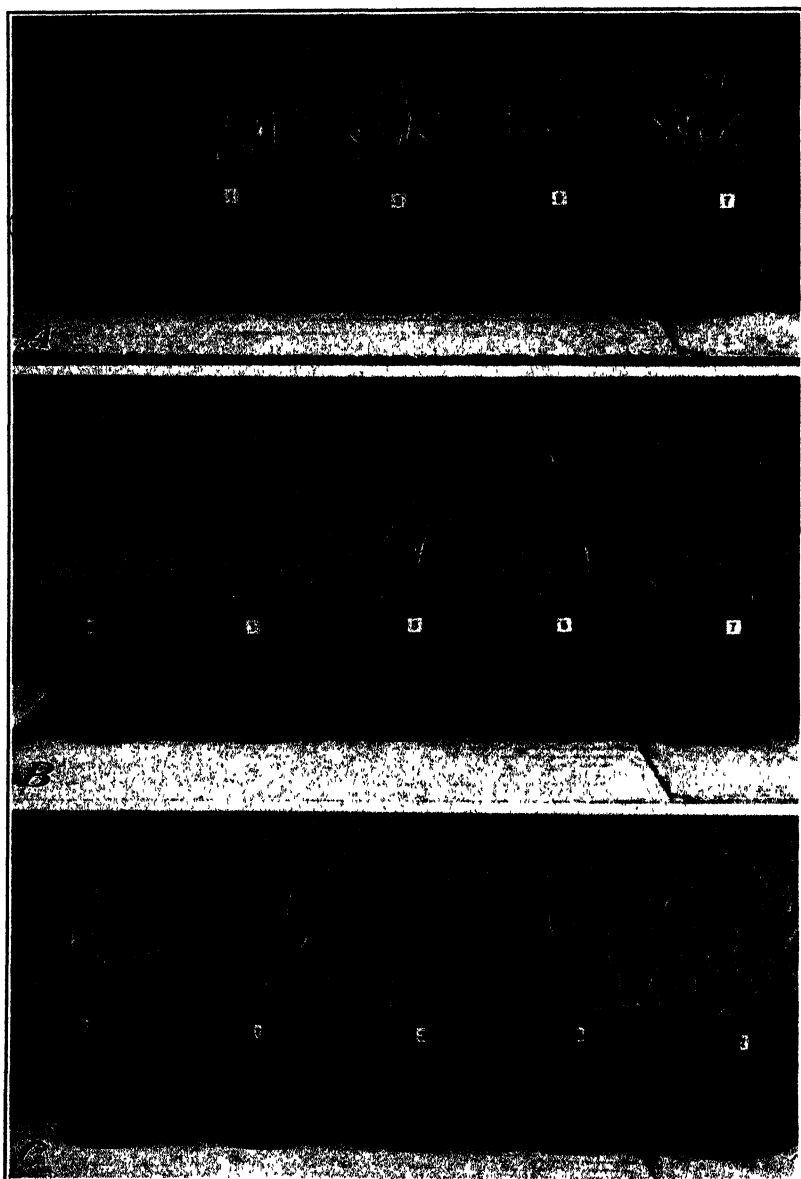


FIGURE 3.—Comparative degrees of inhibition of selenium (20 p. p. m.) injury by various amounts of sulphur in two different soils. Numbers on pots indicate grams of sulphur added to 5,000 g of soil. (Abnormally rapid development of the plants was due to lateness of the season, the grain having been sown April 28): *A*, One-month-old plants in Pierre clay injured by selenium but progressively improved as the amount of added sulphur was increased; *B*, same plants as in *A* 1 month later, dead with no sulphur treatment, severely injured with but 1 g, slightly injured with 3 g, and almost normal with 5 and 7 g; *C*, 2-month-old plants in Keyport clay loam, severely injured with no sulphur treatment, slightly chlorotic with 1 g, uninjured with 5 and 7 g.

for prevention of the symptoms produced by the addition of 20 p. p. m. selenium as sodium selenate to the two soils, Pierre clay and Keyport clay loam, are shown in figure 3. In the former soil the addition of 20 p. p. m. of selenium was fatal without the application of sulphur (*A, B*), but in the latter soil it was merely injurious (*C*). As the quantity of sulphur applied to the Pierre clay series was increased from 1 to 7 g, symptoms of injury were progressively reduced. In the Keyport clay loam, where sodium selenate was less available to the plants (table 2), less sulphur was required for a given degree of control.

Table 3 gives analyses of plants of various soil experiments dealing with the control of selenium injury by sulphur. These analyses show that inhibition of injury is accompanied by a reduction in the quantity of selenium taken into the plant. In every experiment the quantities of selenium taken up and the resulting degrees of injury bore an inverse relation to the amount of sulphur added to the soil.

TABLE 3.—*Effect of elemental sulphur applications on absorption of selenium by wheat plants from sodium selenate added to Keyport clay loam and Pierre clay in the greenhouse (second sowings)*

Type of soil	Selenium added to soil	Sulphur added to soil		Selenium in plants	Degree of selenium injury to plants
		Per pot of 6,000 g	Rate of application		
	<i>P p m</i>	<i>Grams</i>		<i>P p m</i>	
Keyport clay loam	20	0		480	++++
		4	1:1,500	360	+
		5	1:1,200	230	0
Pierre clay	5	0		450	+
		10	1: 600	150	0
Do.	10	0		960	+++
		5	1:1,200	425	0
Do	20	0		1,350	+++++
		2	1:3,000	850	++
		6	1:1,000	800	+
		15	1: 400	600	0

Gypsum (CaSO_4) also was effective in reducing the selenium taken up by wheat plants, as shown by various pot tests and also by some results obtained with field plots at the Arlington Experiment Farm. The soil in these plots was treated in the fall with sodium selenate, at the rate of 2 p. p. m. of selenium, mixed in to a depth of 6 inches. Two of them received elemental sulphur also, at the rate of 1 part sulphur to 1,200 parts soil, and two others received quantities of gypsum containing the same amount of sulphur. Winter wheat was then sown. When sampled the following spring the plants contained from 50 to 110 p. p. m. of selenium except where sulphur had been added. For these the corresponding figures were 7.5 and 5 p. p. m. where the sulphur had been added as gypsum, and 2 p. p. m. for each of the plots that had received elemental sulphur. Evidently, both elemental sulphur and gypsum are effective in inhibiting selenium absorption in the field as well as under greenhouse conditions.

The fact that gypsum was so effective in reducing absorption of selenium precluded any possibility that the inhibiting action of elemental sulphur was a consequence of its acidification of the soil. Other evidence that the phenomenon occurred independently of the

acidifying effect was the complete control of the severe symptoms produced by 5 p. p. m. of selenium in the alkaline Pierre clay by the addition of an amount of sulphur (1 part in 600 parts of soil) which only slightly increased its acidity, that is, from pH 8.1 to 7.9 in one experiment and from 8.3 to 7.8 in another.⁶ Moreover, rendering the Keyport clay loam alkaline by adding calcium carbonate (1 part in 500 parts soil) did not lessen the effectiveness of sulphur applications. Further evidence of the unimportance of acidity in selenium-sulphur antagonism was obtained incidentally in numerous experiments conducted with nutrient solutions, some of which are referred to in a previous publication (7).

To determine whether excess sulphur reduces absorption of naturally occurring selenium as well as that added as sodium selenate, plants were grown in the greenhouse in an untreated Pierre clay loam⁷ containing 4 p. p. m. of naturally occurring selenium. Others were grown with elemental sulphur added at the rate of 1 part in 1,200 parts of this soil. The plants in the untreated soil were found to contain 450 p. p. m. of selenium, those in the sulphur-treated soil 15 p. p. m. Analyses of plants from other pots by M. J. Horn with the colorimetric codeine-sulphate method (5) indicated a similar reduction.

SELENIUM TOXICITY WITH DIFFERENT ADMIXTURES OF SAND TO SOIL

The addition of 20 percent of quartz sand to the local Keyport clay loam increased the toxicity of sodium selenate to the extent that 10 p. p. m. of selenium, an otherwise harmless concentration in this soil (fig. 2, *A*), produced severe chlorosis and stunting (fig. 2, *C*). Likewise, 30 p. p. m., an injurious but not ordinarily fatal concentration, killed the plants where the sand had been added.

This increased severity of injury in sandy soil was further demonstrated by an experiment in which different percentages of sand were mixed with the clay loam. Sodium selenate at the rate of 20 p. p. m. of selenium by weight was added to all with thorough mixing. Where no sand was added the young plants after 3 weeks showed but an occasional trace of chlorosis; with one-fifth sand there was distinct whitening at the base of all the young leaves; with two-fifths sand chlorosis was much more severe and the plants were stunted; with three-fifths sand the chlorotic leaves were a deep rose pink at the base and extremely stunted, emergence of the second leaf being inhibited; and with four-fifths sand the plants turned yellow and died shortly after emerging from the soil. The occurrence of the pink color on the plants in the very sandy soil agreed with previous observations that plants in sand cultures frequently showed this coloration, while those in soil did so but rarely (7).

RETENTION OF SELENIUM BY SOIL AS SHOWN BY TOXICITY STUDIES WITH WHEAT

In interpreting the results of some experiments on possible effects of soil moisture on selenium toxicity, it was necessary to know whether the selenium of a sodium selenate solution becomes uniformly distributed throughout a mass of soil on the surface of which it is poured. To determine this, 600 cc of a solution of sodium selenate,

⁶ The acidity determinations were made with the hydrogen electrode immersed in 1:1 and 1:2 suspensions of the soil.

⁷ Analyses by J. B. Martin showed but 0.085 percent total sulphur and 0.002 percent water-soluble sulphur in this soil

enough to supply 20 p. p. m. of selenium by weight of soil, was poured on the surface of 6,000 g of slightly moist soil in a 10-inch pot. The volume of solution was sufficient to penetrate to the bottom of the soil mass, which was approximately 5 inches deep. After standing for several days this soil was transferred in seven successive layers to as many small pots. Then all were brought to the same moisture content and sown with wheat. The relative amounts of selenium reaching each layer were shown by the different degrees of injury that appeared on the plants in the seven small pots. The condition of the plants 3 weeks after sowing, when the third leaf was emerging, was as follows:

Layer no. 1 (top layer of original soil mass)—plants stunted and yellow; too much injured to show white chlorosis on leaves.

Layer no. 2—chlorosis on all leaves; moderate stunting.

Layer no. 3—chlorosis first appearing at base of third leaf; slight stunting.

Layer no. 4—traces of chlorosis; no stunting.

Layers nos. 5-7—normal.

Apparently the selenium did not all penetrate with the water to the bottom of the original soil mass, there being no evidence of toxicity below the fourth layer.

To determine whether soil containing sand would be more penetrable to selenium, the experiment was repeated with 500 cc of a sodium selenate solution, supplying 40 p. p. m. of selenium by weight of soil, poured on 6,000 g of Keyport clay loam in each of four pots, two of which contained an admixture of 20 percent sand. The 500 cc volume of solution just penetrated to the bottom of the soil mass. The experiment was duplicated with the same quantity of selenate added in a volume of 1,000 cc, which made the bottom layers very wet and assured more than ample opportunity for thorough penetration. After standing 2 days the soil in each pot was layered as before into seven small pots. To determine the extent to which the water had penetrated the soil mass under the different conditions, these layers were sampled for moisture content. Then, after equalizing their moisture content, the relative selenium content of each layer was demonstrated by its toxicity to wheat seedlings. The data, together with observations on the condition of the seedlings at the age of 3 weeks, are reported in table 4.

The data in table 4 show that without the addition of sand the selenium did not penetrate below the third layer of the soil in sufficient amount to injure the seedlings. With the addition of sand, enough selenium to produce chlorosis penetrated to the fifth layer. The top layer always contained enough to produce fatal injury (fig. 4).

That the amount of selenium in the layers was not simply determined by the penetrability of the original soil mass to water was apparent from the lack of correlation between the degrees of injury and the moisture percentages of the layers. Distribution of the water was very uniform in the upper layers of both soils, especially where but 500 cc was used, yet the plants ranged from a dying condition in the top layer to normal or, in the sandy soil, nearly normal growth in the fourth. Furthermore, doubling the quantity of water in which the selenium was added, thereby mechanically aiding penetration and greatly increasing the amount of water that reached the lower soil layers, did not increase their toxicity.

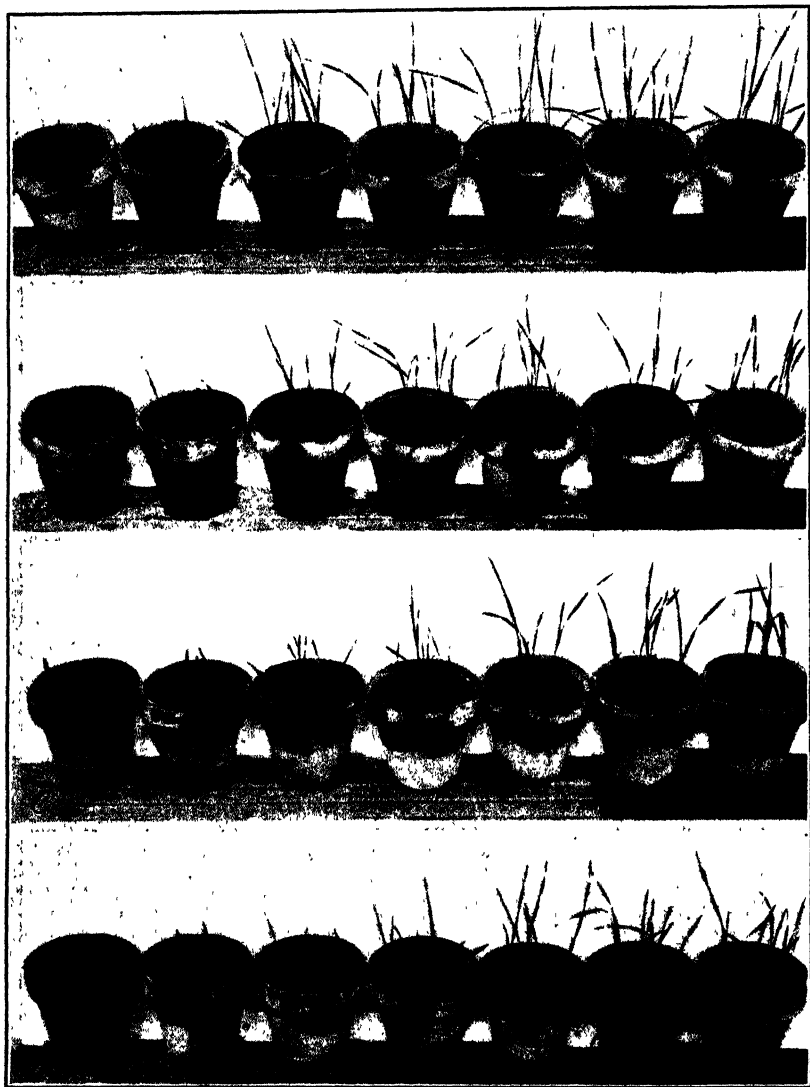


FIGURE 4.—Comparative growth of wheat seedlings in each of seven soil layers taken from top (left) to bottom (right) of four lots of soil (*A* to *D*) on the surface of each of which had been poured a solution of sodium selenate. The selenium had been added to the clay loam soil in volumes of 500 cc (series *A*) and 1,000 cc (series *B*), and to clay loam plus sand in volumes of 500 cc (series *C*) and 1,000 cc (series *D*).

TABLE 4.—Retention of selenium by soils as shown by the comparative toxicity to wheat of the selenium contained in successive layers of a soil mass on the surface of which a solution of sodium selenate was poured

Soil layer (numbered from top)	Keyport clay loam flooded with 0.56 g of selenate dissolved in—						Keyport clay loam plus 20 percent of sand flooded with 0.56 g of selenate dissolved in —					
	500 cc			1,000 cc			500 cc			1,000 cc		
	Condition of plants	Leaf chloro- rosis	Initial mois- ture	Condition of plants	Leaf chloro- rosis	Initial mois- ture	Condition of plants	Leaf chloro- rosis	Initial mois- ture	Condition of plants	Leaf chloro- rosis	Initial mois- ture
1	Dying	---	Pet 14 27	Dying	---	Pet 20. 56	Dying	---	Pet 12. 25	Dying	---	Pet 12. 25
2	Severely stunted	+++	14 54	Severely stunted	+++	18. 72	Severely stunted	+++	12. 14	Severely stunted	+++	12. 14
3	Slightly stunted	+	14. 07	Slightly stunted	+	18. 19	do	+++	12. 76	do	+++	12. 76
4	Normal	0	14. 10	Normal	0	17. 99	Slightly stunted	++	12. 57	Slightly stunted	++	12. 57
5	do	0	13. 65	do	0	17. 25	Normal	+	12. 43	Normal	+	12. 43
6	do	0	13 14	do	0	16. 76	do	0	11. 45	do	0	11. 45
7	do	0	11 75	do	0	16. 49	do	0	10. 91	do	0	10. 91

Other evidence of retention of selenium by the soil was obtained on comparing the toxicity of a sodium selenate solution to wheat seedlings before and after filtering through Keyport clay loam. Two and a half liters of a highly toxic solution containing 200 p. p. m. of selenium was poured on the surface of 5,000 g of soil closely packed in a soil cylinder 30 cm high and 15 cm in diameter. The solution dripped through slowly, and five 100-cc portions were collected over an interval of 3 days. Each 100-cc portion was diluted to 1.5 l with water and poured on 7,000 g of quartz sand in a pot, which was then sown with wheat. None of the seedlings showed chlorosis or other symptoms of injury. Control pots received an equal volume of a solution containing 100 cc of an equivalent water extract of untreated soil (to provide the same quantities of sulphates and other soluble constituents) in addition to 100 cc of the selenate solution not filtered through the soil. The seedlings in these pots all became chlorotic. The absence of symptoms of toxicity where the selenium solution had been filtered through the soil was attributed to the disappearance of at least some of the selenium. It was concluded that when a solution of sodium selenate is poured on the surface of this soil the selenium does not all penetrate with the water in which it is dissolved but is at least partly retained in the upper layers.

COMPARATIVE ABSORPTION OF DIFFERENT FORMS OF SELENIUM

Elemental selenium is not readily if at all available to the wheat plant. When added to the Keyport clay loam in quantities up to 200 p. p. m. it produced no sign of toxicity and no trace of it could be found in plants tested by the codeine-sulphate method.

When compared on the basis of equal concentrations of selenium, sodium selenate was decidedly more toxic than sodium selenite. When 30 p. p. m. of selenium was added to the Keyport clay loam as sodium selenate, wheat plants were chlorotic, extremely stunted, and contained 1,000 p. p. m. of selenium, but when it was added as sodium selenite the plants were uninjured and contained but 10 p. p. m. of

selenium. In fact, the plants were not injured by concentrations as high as 70 p. p. m. of selenium added in the form of the selenite and analyses showed absorption of but 20 p. p. m. by weight of the tissues. A corresponding difference in absorption from the two salts was shown also by analyses of plants from other experiments. It is quite possible that the difference is attributable to rapid reduction of the selenite to elemental selenium in the soil.

EXHAUSTION OF SELENIUM FROM THE SOIL BY SUCCESSIVE CROPPINGS

Successive sowings of wheat in the same selenized soil were progressively less injured, indicating that sodium selenate was either reduced to a subtoxic concentration by the growth of the successive crops or changed to a less toxic form of selenium, possibly by the activity of microorganisms (8). Initial concentrations as high as 30 p. p. m. of selenium, which almost killed the plants of the first sowing, produced no visible effect on the plants of the fourth. In table 5 are recorded the relative degrees of chlorosis and stunting (indicated by number of plus signs) shown by four successive crops of wheat (five plants per pot) grown to maturity in 4,000 g of selenized soil. The soil remained without further treatment after the initial admixture of sodium selenate except for the addition of potassium nitrate when the plants began to show nitrogen deficiency.

TABLE 5.—*Progressively decreased toxicity of sodium selenate for successive crops of wheat in the same soil*

[Each crop consisted of 5 plants per pot]

Crop	Relative degrees of injury to wheat plants from the following concentrations of selenium as sodium selenate		
	5 p. p. m.	15 p. p. m.	30 p. p. m.
First.....	0	+++	++++
Second.....	0	++	+++
Third.....	0	0	+
Fourth.....	0	0	0

DISCUSSION

In the naturally seleniferous areas of the Middle West (2, 11, 13) the vegetation is very toxic to animals, yet shows no external symptoms of injury (3). In some of these areas surprisingly large accumulations of selenium in the plants are reported (1). The data presented in the present paper have shown that large quantities of selenium can be taken up by the plant before external symptoms of abnormality appear. Thus 380 p. p. m. was found in the leaves of plants grown in the Keyport clay loam to which had been added 10 p. p. m. of selenium (table 2), yet the plants showed no visible injury. Similarly, in the Pierre clay 325 p. p. m. was absorbed from 1 p. p. m. in the soil without visibly affecting the plants. This ability of the plant to accumulate selenium accounts for the toxicity to experimental animals of normal-appearing wheat grown with but 1 p. p. m. of selenium added as sodium selenate to the local clay loam (10).

Where excess sulphur is available still larger quantities of selenium can be taken up by the plants without injury. Those grown in Pierre clay to which was added 15 g of sulphur per 6,000 g of soil grew normally with 600 p. p. m. absorbed into their tissues from 20 p. p. m. of selenium in the soil, yet other plants on selenized soils with less sulphur were injured by lesser accumulations (table 3). Thus it is not possible to predict the selenium content of the tissues by their appearance, nor to establish any particular critical concentration within the plant which when exceeded will result in injury. Under the conditions of the present experiments some plants showed slight chlorosis with but a little over 300 p. p. m. of selenium in the tissues and others showed no chlorosis with twice this amount.

These experiments have suggested that whether or not the presence of a given amount of selenium results in visible injury depends on the amount of sulphur taken up to act as a protective agent by decreasing the chances of substitution of selenium for sulphur in molecules susceptible to such substitution. This explanation is based on the theory suggested previously to explain the selenium-sulphur antagonism (7). The theory assumes that because of their chemical similarity the plant is unable to distinguish between selenium and sulphur. While the total selenium plus sulphur absorbed by the plant would increase with that in the substratum, the proportionate amount of selenium in this total would be the same as that in the external medium. The relative number of selenium ions with reference to sulphur ions available within the plant would determine the proportionate number of "susceptible" molecules whose sulphur is replaced by selenium, and, consequently, the degree of injury to the plant. The excess selenium not so substituted might reasonably be supposed to occur in the plant as selenate (just as excess sulphur occurs as sulphate), incapable of harming the plant so long as excess sulphur sufficiently reduces the chance of substitution.

If these assumptions are correct, plants having excess sulphur available should absorb without injury quantities of selenium which would be fatal at lower sulphur levels. The data available indicate that they do. Moreover, the capacity for selenium absorption by a given crop should vary with the capacity for sulphur absorption and utilization characteristic of the species. That this generalization also obtains is suggested by the data in table 1, which show that the greatest absorption of selenium occurred in the two species of the Cruciferae, the least in representatives of the Gramineae, and that the legumes were intermediate. The three groups stand in this same order with respect to their sulphur requirements (4, 9).

SUMMARY

Under the conditions of these experiments, the following factors have been found to affect the absorption and resulting toxicity to wheat of selenium added as sodium selenate to the soil: Available sulphur, soil type, percentage of sand, method of adding selenium, the form of selenium added, and growth of previous crops.

Of 17 different crops grown in Keyport clay loam with 5 p. p. m. selenium added as sodium selenate, the Cruciferae, broccoli and mustard, absorbed more selenium than any others, i. e., 1,180 and 1,240 p. p. m., respectively. It is suggested that the tendency of a crop to absorb selenium depends on its tendency to absorb sulphur.

Sodium selenate is more easily absorbed by wheat from Pierre clay than from Keyport clay loam. The greatest accumulation was 1,350 p. p. m., found in plants fatally injured by 20 p. p. m. of selenium as sodium selenate added to Pierre clay.

Elemental sulphur applications reduce the absorption by wheat of the naturally occurring selenium in the soil, as well as of that added as sodium selenate. Gypsum is similarly effective.

The addition of quartz sand to Keyport clay loam increases the toxicity of sodium selenate in proportion to the percentage of sand in the mixture.

Sodium selenate is not easily leached from Keyport clay loam, being at least partially retained in the upper layers when a solution containing it is poured on the surface. Solutions originally toxic are nontoxic after being filtered through this soil. The presence of sand increases penetrability.

Elemental selenium is apparently unavailable and nontoxic to wheat plants, at least in quantities up to 200 p. p. m. in Keyport clay loam.

The selenium in sodium selenate is more available and more toxic to wheat than that in sodium selenite, the comparison being made on the basis of equal concentrations of selenium.

Sodium selenate was either changed to a less toxic form of selenium or reduced to a subtoxic concentration by the growth of successive crops of wheat.

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DORMANCY AND MATURITY OF COTTONSEED ¹

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INTRODUCTION

In studying the moisture content and germination of cottonseed during the period of boll opening at the United States Acclimatization Field Station, near Charleston, S. C., it was found that germination tests made on the seed immediately after they were harvested were unreliable. The fresh seed germinated very slowly, and many seed, though apparently sound, failed to germinate even after being in the germination chambers for 24 days. However, when fresh seed was thoroughly air-dried and stored for a few weeks, better germination was obtained.

DORMANCY OF FRESH SEED

In 1932, seed samples of several cotton varieties were studied to determine the moisture content and the percentage germination immediately after harvesting and after drying and storing for 1 month. Data in table 1 show the degree of dormancy of fresh seed of bolls open from 1 to 10 days of the upland varieties Tidewater, Cleveland, and Foster, and of the Seabrook strain of sea island.

The fresh seed of all the upland varieties from bolls open 1 to 5 days germinated very slowly, and a considerable percentage of sound or dormant seed remained ungerminated after being in the germination chambers for 24 days; whereas complete germinations usually are obtained from normal dry seed in from 5 to 7 days. While more rapid germination and higher total percentages of germination were obtained from fresh seed that had remained in the field more than 5 days, the rate of germination was not so rapid as that of seed which had been dried and stored, nor was the seed entirely free from dormancy. In practically every case, the germination percentage of the seed which was dried and stored for 1 month was higher than that of fresh seed of the same sample. In the case of seed from bolls of the upland varieties open only 1 and 2 days, drying and storing increased the germination percentages by an average of 56.4 percent. There was no appreciable difference in dormancy of fresh seed among the several upland varieties.

¹ Received for publication Sept. 25, 1934, issued May 1935.

² The writer is indebted to B. M. Stone, formerly assistant scientific aid, Division of Cotton and Other Fiber Crops and Diseases, for the collection of many of the data presented in this paper.

TABLE 1.—*Dormancy of cottonseed during the period of boll opening at the United States Acclimatization Field Station, James Island, S. C., 1932*

TIDEWATER (TAGGED SEPT. 7)

Bolls picked	Per- cent- age of mois- ture in seed	Percentage of seed germinated after designated number of days in germinator																								Per- cent- age of sound but un- germi- nated seed	Percentage of seed germinated after being dried and stored about 1 month, and after design- ated number of days in germinator		
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total	5	7		Total		
Sept. 7	52.0	---	---	---	---	---	---	---	---	13	---	6	---	---	---	---	11	---	2	---	---	---	32	30	84	7	91		
8	43.0	---	---	---	---	21	---	---	10	---	---	---	---	---	---	11	---	1	---	---	3	---	46	27	90	3	93		
9	34.7	---	---	17	---	---	14	---	---	---	---	---	---	10	---	---	4	---	2	---	---	---	51	27	90	1	91		
10	31.2	---	---	---	43	---	---	---	5	---	---	---	---	---	---	5	---	---	---	---	---	---	53	7	85	2	87		
11	13.0	---	---	---	59	---	12	---	---	---	3	---	---	---	---	---	---	---	---	---	---	---	74	2	93	3	96		
12	12.1	---	---	60	---	12	---	---	3	---	1	---	---	---	---	---	---	---	---	---	---	---	76	0	93	3	96		
13	11.2	---	---	79	---	7	---	---	9	---	---	---	---	---	---	---	---	---	---	---	---	---	81	3	95	2	97		
14	27.2	---	---	---	---	---	6	---	8	---	---	---	---	---	---	---	---	---	---	---	---	---	87	3	85	5	90		
15	34.0	---	---	63	---	---	6	---	3	---	---	---	---	---	---	1	---	---	---	---	---	---	73	12	92	1	93		
16	23.3	42	18	---	16	---	3	---	1	---	---	---	---	---	---	---	---	---	---	---	---	---	80	0	85	2	87		

CLEVELAND (TAGGED SEPT. 7)

Sept. 7	48.7	---	---	---	---	---	13	---	---	6	---	---	---	---	11	---	2	---	---	---	---	---	32	30	54	19	73
8	38.9	---	---	---	3	---	9	---	---	---	---	---	5	---	3	---	---	---	7	---	---	---	27	59	75	12	87
9	29.7	---	8	---	13	---	---	---	---	---	8	---	---	---	3	---	2	---	---	---	---	---	42	39	59	29	88
10	17.3	---	---	17	---	7	---	---	---	---	11	---	---	---	---	---	---	---	---	---	---	---	35	17	41	44	85
11	15.9	---	---	10	---	18	---	---	10	---	---	---	---	---	8	---	---	---	---	---	---	---	46	21	64	20	84
12	13.3	---	12	---	15	---	24	---	10	---	---	---	---	---	---	---	---	---	---	---	---	---	61	13	55	39	94
13	12.7	---	---	14	---	---	27	---	8	---	7	---	---	---	---	---	---	---	---	---	---	---	56	26	73	14	87
14	27.5	---	21	---	---	41	---	4	---	1	---	---	---	---	---	---	---	---	---	---	---	---	67	17	90	7	97
15	38.7	---	15	---	18	---	---	---	---	2	---	---	---	---	---	---	---	---	---	---	---	---	35	42	70	17	87
16	22.8	5	14	---	13	---	9	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	49	15	75	6	81

FOSTER (TAGGED SEPT. 7)

Sept. 7	50.9	---	---	---	---	5	---	4	---	26	---	11	---	---	---	---	---	---	---	---	---	---	46	41	95	0	95
8	41.0	---	---	---	1	---	2	---	---	20	---	12	---	---	---	---	---	---	---	---	---	---	42	35	94	1	95
9	28.5	---	10	---	13	---	---	---	10	---	2	---	---	---	---	---	---	---	---	---	---	---	43	22	78	16	94
10	16.0	---	---	56	---	6	---	---	---	---	6	---	---	---	---	---	---	---	---	---	---	---	68	6	88	8	96
11	12.7	---	24	---	23	---	10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	58	11	54	42	96
12	10.0	---	27	---	27	---	10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	61	4	63	30	93
13	11.3	---	---	30	---	39	---	5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	74	12	91	3	97
14	28.3	---	33	---	49	---	2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	84	4	76	20	96
15	35.9	---	25	---	40	---	4	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	73	8	83	11	94
16	21.6	9	8	35	13	---	4	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	69	8	77	10	87

TIDEWATER (TAGGED SEPT. 20)

Sept. 20	54.1	---	---	---	19	---	---	4	---	---	---	---	---	---	6	---	4	6	---	---	---	---	32	42	188	9	97
21	51.7	---	---	---	6	---	15	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	32	50	196	1	97
22	45.6	---	10	---	7	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	35	32	172	17	89
23	38.2	14	---	19	---	16	---	14	---	---	---	---	---	---	5	---	3	---	3	---	---	---	58	22	192	4	96
24	31.0	---	43	---	7	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	67	4	184	7	91
25	24.9	---	34	---	25	---	13	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	76	0	184	6	90
26	27.0	---	49	---	7	---	4	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	60	3	179	2	81
27	28.6	---	45	---	14	---	5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	64	0	184	2	86
28	22.5	34	19	---	5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	60	0	180	12	92
29	(²)	42	24	---	6	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	72	2	176	2	78

¹ After 4 days in germinator.² Moisture content of seed was not determined.

TABLE 1.—*Dormancy of cottonseed during the period of boll opening of the United States Acclimatization Field Station, James Island, S. C., 1932—Continued*

CLEVELAND (TAGGED SEPT 20)

Bolls picked	Per- centage of mois- ture in seed	Percentage of seed germinated after designated number of days in germinator																								Per- centage of sound but un- germi- nated seed	Percentage of seed germinated after being dried and stored about 1 month, and after design- ated number of days in germinator		
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total	5	7		Total		
Sept 20	48.8	-	-	-	-	12	-	-	-	-	8	-	-	-	-	-	-	6	-	-	-	4	30	53	184	9	93		
21	47.9	-	-	-	-	12	-	-	10	-	-	-	-	9	-	-	13	-	6	-	-	50	42	169	20	89			
22	46.3	-	9	-	-	5	-	-	11	-	-	-	-	-	-	6	-	-	-	-	-	31	53	172	18	90			
23	34.0	17	-	-	-	21	-	-	2	-	-	-	-	8	-	-	6	-	2	-	-	56	27	165	32	97			
24	30.1	-	14	-	-	28	1	-	-	6	-	-	-	9	-	-	-	-	-	-	-	58	22	167	26	93			
25	26.7	-	16	-	-	21	-	23	-	-	-	-	-	-	3	-	-	-	-	-	-	63	15	184	5	89			
26	28.7	-	-	-	29	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	45	0	165	8	73			
27	31.1	-	16	-	26	14	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	59	0	157	20	77			
28	27.1	-	17	-	28	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	55	0	166	10	76			
29	(1)	3	37	-	22	8	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	71	9	148	25	73			

FOSTER (TAGGED SEPT 20)

Sept 20	50	1																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													</
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TIDEWATER (TAGGED OCT. 5)

Oct 5	53.9	-	-	3	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	5	50	79	1	80
6	53.1	-	-	2	-	7	-	-	-	-	10	-	-	5	-	-	-	-	-	-	-	24	53	82	6	88
7	52.2	-	2	-	5	7	2	-	-	-	-	-	3	-	-	-	-	-	-	-	-	16	73	77	6	83
8	48.2	-	3	-	-	-	-	-	-	6	4	-	-	-	-	-	-	-	-	-	-	21	67	87	1	88
9	33.0	-	3	-	6	-	-	9	5	-	-	-	-	-	-	-	-	-	-	-	-	23	61	87	5	92
10	37.8	-	-	-	19	-	-	4	2	-	-	-	-	-	-	-	-	-	-	-	-	25	56	86	3	89
11	24.5	-	33	-	11	-	9	-	3	-	-	-	-	-	-	-	-	-	-	-	-	56	14	81	9	90
12	15.8	10	-	-	57	3	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	77	11	85	9	94
13	11.3	31	-	-	47	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	85	7	93	4	97
14	13.7	10	18	-	38	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	78	12	78	6	84

SEA ISLAND (TAGGED OCT. 5)

Oct 5	48.4	-	-	45	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	92	0	196	0	96
6	48.2	-	70	-	24	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	95	0	197	0	97
7	49.0	54	-	31	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	93	0	195	0	96
8	41.2	-	99	-	21	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	91	0	194	1	95
9	29.4	-	49	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	97	0	196	0	96
10	20.2	89	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	0	197	1	98
11	17.1	-	87	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	0	191	6	97
12	13.9	95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95	0	192	0	92
13	12.3	89	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	91	0	192	0	92
14	13.8	96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	93	0	197	0	97

1 After 4 days in germinator.

2 Moisture content of seed was not determined.

From the differences in germination of fresh and dried seed, it is evident that the percentage of sound seed remaining in the germinator after 24 days did not represent the total percentage of dormant seed. The results indicated that many seed which rotted under the conditions of high temperature and moisture in the germinator would have germinated if they had been dried and stored for a short period.

The total germination percentages of both fresh and dried seed were affected by the length of time during which the seed had been exposed previously in the field, but some degree of dormancy was evident even in seed from bolls harvested 10 days after opening began. When the seed was dried and stored, higher total germinations usually were obtained from the seed which was harvested when the bolls were just beginning to open and before any field deterioration could take place than from bolls exposed for a longer period in the field. This was particularly evident in samples from bolls opening during periods of wet weather, as from September 20 to 29, rain being recorded on 6 days of this period.³

The behavior of fresh sea-island seed as regards dormancy was in striking contrast to that of the upland varieties. The comparison between the Tidewater and sea island given in the latter part of table 1 shows that the fresh seed of the sea island required only a few days more to germinate than the dried seed and that the total germination percentages were only slightly lower for fresh seed than for seed which was dried and stored for 1 month. Of the seed from sea-island bolls harvested on the first day after opening began, 85 percent germinated in 7 days and 92 percent in 10 days. Of the Tidewater seed harvested at the same time only 5 percent germinated in 24 days, but in the sample that was dried and stored 80 percent germinated in 7 days.

Since dormancy of fresh seed may be eliminated by drying and storing for a short period, it is of interest to know whether drying alone will give equally good results. To obtain information on this point, 20 bolls of Tidewater cotton, one-fourth inch open at the tips, were picked on October 18 and divided into 3 samples. The seeds were removed from sample 1 and placed in the germinator immediately. Samples 2 and 3 were thoroughly dried, and on October 24 the seeds of sample 2 were placed in the germinator. Sample 3, after being dried, was placed in storage and kept there until November 18, when it was placed in the germinator. The germination percentages for these 3 samples were 12 for the fresh undried seed, 75 for the dried unstored seed, and 84 for the dried seed stored for 30 days. The fresh undried seed continued to germinate for 21 days; 59 percent of the dried unstored seed germinated in 7 days and germination was complete in 12 days. Seventy-two percent of the seed of sample 3, which was dried and stored, germinated in 5 days and 84 percent in 7 days. These data indicate that, while a part of the dormancy may be removed by drying alone, further improvement in the germination of the seed is obtained by short storage after drying.

SEED MATURITY

Experiments had indicated that seed from bolls just beginning to open, if properly dried and stored, would give high germination percentages and that the failure of these seed to germinate immedi-

³ See table 1 of the following publication: SIMPSON, D. M., and STONE, B. M. VIABILITY OF COTTONSEED AS AFFECTED BY FIELD CONDITIONS. *Jour. Agr. Research* 50: 435-447. 1935.

ately after harvesting was due to dormancy and not to immaturity of the seed.

In order to determine at what age in the development of the boll the seed becomes germinable, an experiment was conducted in 1932 in which seed from bolls of known age was used. Samples of bolls of Tidewater cotton were collected 21 to 56 days after flowering. The seed cotton was removed from these bolls at the time of harvesting and sun-dried to remove excess moisture. The samples were then stored for approximately 1 month before the germination tests were made. Seed from bolls 21 to 42 days old failed to germinate. Sixty-eight percent of seed from bolls 46 days old germinated, and higher germination percentages were obtained as the percentage of open bolls increased. Bolls began opening about 48 days after flowering and all bolls were open 51 days after flowering. Germination percentages at 52, 54, and 56 days after flowering were 86, 81, and 91, respectively.

Further information on seed maturity was collected in 1933, the data in table 2 being obtained on four varieties of upland cotton at three periods during the season. Ten percent of the seed of the Foster variety from flowers tagged on June 15 germinated at 40 days from fertilization, while seed of other varieties failed to germinate at 40 days. A few seed in all varieties germinated at 42 days from fertilization, and normal germination percentages of seed of all varieties were obtained 48 to 50 days after flowering.

TABLE 2.—Germination of seed from bolls of four varieties of cotton harvested from 40 to 50 days after flowering, James Island, S. C., 1933

FLOWERS TAGGED JUNE 15

Days after flowering (number)	Percentage germination of seed				Remarks
	Tide- water	Coker Wilds	Foster	Cleve- land	
40	0	0	10	0	
42	11	7	12	16	
44	28	24	47	27	2 bolls of Foster open.
46	33	9	37	90	1 Tidewater, 1 Foster, and all Cleveland open
48	65	80	90	83	2 Tidewater, 1 Coker Wilds, all Foster and Cleveland open.
50	95	79	68	89	All bolls open.

FLOWERS TAGGED JUNE 22

40	3	12	25	13	
42	36	55	68	71	1 Tidewater, 2 Foster, and 4 Cleveland open.
44	83	84	87	75	2 Tidewater, 3 Coker Wilds, and all Cleveland open.
46	87	74	97	77	3 Tidewater, 3 Coker Wilds, 6 Foster, and all Cleveland open.
48	71	76	86	87	4 Tidewater and all others open.
50	67	95	93	94	All bolls open.

FLOWERS TAGGED JULY 7

40	68	42	74	36	
42	45	39	66	60	
44	67	64	74	68	
46	57		57	76	
48	74				

Bolls from flowers tagged on June 22 and July 7 were progressively earlier in maturing seed. This would be expected, as the warmer weather of midsummer hastened the maturity of the bolls. Some irregularities in individual germination percentages and in the final total are due to sampling and to field deterioration of seed in the open bolls.

SUMMARY

Experiments conducted at James Island, near Charleston, S. C., indicate that freshly opened cotton bolls contain a considerable percentage of dormant seed. This dormancy may be eliminated by drying and storing the seed for a short period. There was no appreciable difference in dormancy of fresh seed among several upland varieties; the sea-island strain tested showed practically no dormancy.

Studies of seed maturity indicated that cottonseed reaches maturity shortly before the bolls begin to open; at James Island, S. C., this period is from 40 to 50 days after flowering.

VIABILITY OF COTTONSEED AS AFFECTED BY FIELD CONDITIONS¹

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INTRODUCTION

Differences in the viability of cottonseed produced in different seasons have been recognized, and agricultural agencies frequently find it necessary to issue precautionary warnings to farmers regarding the low viability of planting seed in certain sections of the Cotton Belt. Although the low viability of the planting seed is generally attributed to seasonal conditions, little information is available as to the causes of seed deterioration or the relation of seasonal conditions to seed viability.

Experiments conducted at the United States Acclimatization Field Station, James Island, S. C., in 1931, 1932, and 1933 indicate definite relations between seed viability and the weather conditions prevailing while the seed cotton is exposed in the field. The climate at James Island is characterized by high humidity and frequent rains during the harvesting season. Such conditions are unfavorable for the production of seed of high germination, but they provide an opportunity for the study of the causes of deterioration which would not be afforded in a section where conditions for the production of good seed more uniformly prevail.

MOISTURE CONTENT AND GERMINATION OF SEED DURING BOLL-OPENING PERIOD

Weather conditions (table 1) prevailing in the coastal section of the Southeastern States during the cotton-picking season necessitate the harvesting of the cotton as rapidly as is practicable after boll opening begins. The cotton is picked at about 10-day intervals if weather conditions permit. While picking at such short intervals prevents undue exposure of cotton in the fields and protects the quality and grade of the fiber, many bolls containing a high percentage of moisture are usually included in the harvested cotton.

Examination of seed cotton as brought in from the field showed that an appreciable amount of the excess moisture was due to the picking of partially opened bolls. Because of this it was desirable to determine the amount of moisture present in the bolls during the opening process and the rate of drying and the viability of the seed of the partially opened bolls.³

Experiments were begun in 1931 to determine the moisture content of the seed cotton and seed and the germination of the seed during

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² The writers are indebted to E. H. Toole, physiologist, Division of Seed Investigations, Bureau of Plant Industry, for suggestions in planning these experiments and for instruction in the technic of seed germination.

³ The extraction of moisture from the various samples of material for determinations of moisture percentages was accomplished by weighing the samples before drying in an electric oven for approximately 24 hours at a constant temperature of 100° C. The samples were then placed in a desiccator to cool before making the final weighing.

the period of boll opening. Two hundred bolls of the Tidewater variety were tagged just as they were beginning to open at the tip. The bolls were tagged on September 15, and, beginning on that date, two 10-boll samples were picked at random each week day to and including September 25. The samples in the first series were used to determine the moisture content of the seed cotton, while those in the other series were ginned and determinations were made of the moisture content and germination percentage of the seed.

TABLE 1.—Maximum and minimum temperatures, relative humidity, and rainfall, from Aug. 1 to Oct. 19, 1931, 1932, and 1933, at the United States Acclimatization Field Station, James Island, S. C.

Date	1931					1932					1933				
	Temperature		Relative humidity at 8 a. m.	Rainfall (24-hour period) recorded at 8 a. m.		Temperature		Relative humidity at—		Rainfall (24-hour period) recorded at 8 a. m.	Temperature		Relative humidity at—		Rainfall (24-hour period) recorded at 8 a. m.
	Maximum	Minimum				Maximum	Minimum	8 a. m.	1 p. m.	5 p. m.	Maximum	Minimum	8 a. m.	1 p. m.	5 p. m.
	° F.	° F.	Pct.	Inches	° F.	° F.	Pct.	Pct.	Pct.	Inches	° F.	° F.	Pct.	Pct.	Pct.
Aug. 1	87.5	70.5	76	—	85.0	77.0	82	—	—	—	89.0	74.0	75	—	—
2	89.0	76.5	—	—	85.5	77.0	74	—	—	—	89.0	72.5	80	—	—
3	89.5	78.0	76	—	89.0	78.0	76	—	—	—	87.0	77.0	80	—	—
4	90.5	76.0	80	—	88.0	80.0	76	—	—	—	88.5	74.0	78	77	0.50
5	90.0	76.0	77	—	85.0	72.0	93	—	—	1.63	88.0	70.0	85	—	.44
6	89.5	68.5	69	—	82.0	73.0	86	—	—	1.40	88.0	71.0	80	72	—
7	87.0	71.5	91	—	87.0	77.0	84	—	—	.07	87.0	72.0	88	64	74
8	89.0	71.0	91	0.45	87.0	74.0	87	—	—	—	87.0	72.0	83	73	76
9	89.5	73.5	—	—	87.0	73.0	85	—	—	.09	89.0	70.5	85	63	63
10	88.0	76.0	83	.61	86.5	73.0	87	83	—	—	90.0	74.5	81	70	—
11	82.5	72.5	—	—	90.5	75.0	80	68	70	.01	92.0	76.0	76	72	.01
12	80.0	63.0	95	1.33	93.0	75.0	80	65	78	—	90.0	75.5	82	—	.77
13	85.5	66.0	85	.09	90.0	73.0	85	69	74	.03	89.0	70.0	87	—	1.74
14	81.0	66.0	93	.53	83.0	75.0	81	48	—	.10	90.5	75.0	79	57	67
15	84.0	70.0	87	.80	84.0	70.0	73	66	72	—	90.0	70.0	78	57	81
16	85.5	73.5	76	—	87.0	69.5	79	—	67	—	86.0	72.5	93	77	83
17	89.5	70.5	76	—	87.0	70.0	70	61	61	—	83.5	72.5	98	80	83
18	86.0	69.5	75	—	82.0	69.0	60	—	—	—	87.5	73.0	91	62	79
19	86.5	73.0	84	—	80.0	73.0	95	85	85	.35	77.5	70.5	95	93	—
20	89.0	74.5	76	—	87.5	73.0	98	81	84	.81	77.5	70.0	98	—	.65
21	87.5	70.0	79	—	80.0	70.5	98	81	83	4.00	77.0	70.0	98	—	.55
22	85.0	69.0	82	—	79.0	70.5	91	87	83	.08	83.0	71.5	78	67	.76
23	77.5	58.0	—	—	82.5	71.5	85	76	—	.04	87.5	69.0	78	75	75
24	85.0	56.0	81	—	82.5	70.5	93	77	77	—	87.0	74.5	79	75	74
25	87.0	61.0	71	—	82.0	70.0	84	79	81	1.32	88.5	73.5	88	73	80
26	85.5	70.0	67	—	84.5	71.0	83	68	81	—	90.0	75.0	84	77	—
27	86.5	68.5	77	—	85.0	72.0	81	69	73	—	92.0	78.0	88	—	.23
28	95.0	66.5	85	—	88.0	70.0	91	66	74	—	91.0	74.0	78	69	.72
29	89.5	73.0	79	.85	89.5	71.5	89	66	68	—	92.5	78.0	80	67	.78
30	86.0	70.0	—	—	89.5	72.5	91	61	84	—	82.0	72.5	98	—	.85
31	85.0	66.0	87	.75	88.5	73.5	88	67	82	—	84.0	71.0	91	76	.60
Sept. 1	86.0	67.0	87	—	89.0	73.5	84	67	80	—	85.5	72.0	89	73	.93
2	85.0	68.0	75	—	92.0	73.0	91	65	61	—	85.0	72.5	85	—	.01
3	88.0	73.0	85	.89	86.0	76.0	73	69	—	—	87.0	81.0	80	—	—
4	88.5	67.0	85	1.47	85.5	72.0	73	63	—	—	88.5	81.0	84	77	.78
5	83.0	70.5	79	.07	86.0	75.5	73	73	—	—	83.0	75.5	82	79	.95
6	87.0	68.0	—	—	88.0	75.0	86	67	78	—	85.5	71.0	98	75	87
7	88.5	73.0	—	—	81.0	72.0	61	40	44	—	85.0	73.0	79	73	.72
8	83.0	72.0	85	—	81.0	55.0	56	34	40	—	88.0	75.0	84	70	.73
9	83.0	63.0	79	—	83.5	53.0	66	26	38	—	90.0	75.0	87	—	—
10	83.0	64.5	82	—	83.0	61.0	75	43	60	—	91.5	74.0	83	—	—
11	84.0	72.5	83	—	81.0	76.0	56	66	49	—	91.5	73.0	83	68	.77
12	84.5	69.5	91	—	82.0	66.0	80	—	76	—	93.5	74.0	86	—	—
13	84.0	71.5	—	—	81.0	70.0	74	62	74	.01	89.0	75.0	84	—	—
14	83.0	68.0	77	.67	74.0	69.0	98	98	94	.80	90.0	75.0	88	—	—
15	84.5	68.5	91	—	73.5	65.0	97	97	95	3.61	90.5	73.0	89	—	—
16	86.5	65.5	98	—	87.0	61.5	72	56	70	1.74	90.5	71.5	78	—	—
17	93.5	68.5	98	—	89.5	67.0	82	—	—	—	92.0	73.5	88	—	—
18	97.0	70.5	62	—	82.0	68.0	91	—	—	—	91.0	72.0	87	—	—
19	85.0	70.5	83	—	83.0	68.0	79	72	—	—	86.0	71.0	82	—	—
20	86.0	74.0	—	—	84.0	75.0	71	72	71	—	93.0	73.0	83	—	—
21	87.5	71.5	91	—	82.0	73.0	86	—	68	.01	84.0	65.0	65	—	—
22	86.0	71.0	80	—	84.0	71.5	82	59	71	—	91.0	67.5	77	—	—

TABLE 1.—Maximum and minimum temperatures, relative humidity, and rainfall, from Aug. 1 to Oct. 19, 1931, 1932, and 1933, at the United States Acclimatization Field Station, James Island, S. C.—Continued

Date	1931					1932					1933								
	Temperature		Relative humidity at 8 a. m.	Rainfall (24-hour period) recorded at 8 a. m.	Temperature		Relative humidity at—		Rainfall (24-hour period) recorded at 8 a. m.	Temperature		Relative humidity at—		Rainfall (24-hour period) recorded at 8 a. m.					
	Maximum	Minimum			Maximum	Minimum	8 a. m.	1 p. m.		5 p. m.	Rainfall (24-hour period) recorded at 8 a. m.	Maximum	Minimum		8 a. m.	1 p. m.	5 p. m.		
			° F.	° F.					Pct.					Inches				° F.	° F.
Sept 23	88.0	69.5	91		82.5	66.0	86	61	64	.05	87.0	62.0	75						
24	89.0	72.0	89		82.0	66.5	89	69			87.0	67.0							
25	87.5	66.5	77		80.0	69.5	95		82		86.0	70.0	87						
26	89.0	76.5	82		74.5	68.0	95	95	93	15	86.5	66.5	93						
27	89.0	67.0			78.0	68.0	98	83	86	26	85.5	69.0	69						
28	77.0	51.0	64		85.0	69.5	91	67	93	35	87.5	69.5	84						.04
29	77.0	54.0	68		78.5	68.5	93	64	77	73	88.0	70.5	75						
30	78.5	56.5	71		76.5	58.5	65	44	45		90.0	69.0	79						
Oct 1	80.0	61.5	83		75.0	57.0	72	51	61		87.5	72.0							
2	81.0	58.5	90		71.0	61.0	78	92	97		84.5	69.0	93						.20
3	80.0	62.5	84		76.5	63.0	95	93	74	1.47	77.0	64.0	76						.01
4	82.0	64.0			79.5	66.0	84	68	74	.25	78.0	61.5	80						
5	82.5	66.5	84		81.0	70.0	91	85	80		79.5	64.5	85						
6	82.0	61.0	98		69.0	54.5	88	54	57	35	75.5	57.5	81						
7	83.0	62.0	96		67.0	48.5	64	40	57		80.0	50.0	83						
8	82.5	66.0	88		74.5	50.5	67	61			78.5	58.0							
9	84.0	68.0	86	04	80.0	63.0	80	63			77.5	61.0	77						15
10	79.0	61.5	82	10	78.0	62.5	90	76	78		75.0	46.5	71						
11	78.5	58.0			83.0	63.0	93	48	74		76.0	51.5	86						
12	84.0	55.5	78		71.0	54.0	73	48	59		78.5	51.5	83						
13	77.0	57.0	75		73.0	50.0	89	52	62		82.0	55.5	97						
14	84.0	59.0	90		76.0	57.0	80	56	68		74.0	59.5	97						
15	84.0	65.5	95		78.0	65.0	93				77.0	61.0							
16	81.0	64.0	97		76.0	67.0	95			59	81.0	68.5	74						.02
17	77.0	47.0	77		79.5	69.0	93			1.59	81.0	68.5	86						.29
18	75.5	51.0			72.0	66.0	88			01	79.0	62.0	73						
19	71.5	49.0	68		84.0	62.5	90				76.0	57.0	69						

The moisture content of the seed cotton (sample 1, table 2) at the time the bolls were just beginning to open was 55.9 percent. The drying of the cotton was relatively slow during the first 4 days of the period, the moisture content remaining above 40 percent until September 18, when a few of the bolls were just beginning to become fluffy. On September 19 practically all bolls were fluffy.

A comparison of the data for moisture content of seed cotton and of seed (sample 2, table 2) shows very little difference in the rate of drying during the first 5 days of the test, or until practically all of the bolls were fluffed out. Beginning September 19, the fluffed lint dried more rapidly than the seed. On September 25, the final date of the test, both seed cotton and seed had practically the same moisture percentage, indicating that moisture in excess of that dependent upon atmospheric conditions had been removed.

The seeds from the partially opened bolls, picked before September 18, germinated very slowly, only a few having germinated after being in the germination chambers for 6 days. The seeds from bolls which were allowed to remain in the field longer germinated more promptly, gave higher total germination percentages, and showed fewer dormant.⁴ This experiment was begun without knowledge of the fact that many seeds in freshly opened bolls are dormant, and germination tests of the seed samples were begun immediately

⁴ SIMPSON, D. M. DORMANCY AND MATURITY OF COTTONSEED. Jour Agr. Research 50: 429-434, 1935.

after picking. Hence the percentage of germination of the seeds from these samples was considerably lower than would have been the case if they had been stored during the period of dormancy.

TABLE 2.—*Moisture and germination percentages in Tidewater cotton during the period of boll opening, in 1931*

[Bolls tagged Sept. 15]

Bolls harvested	Relative humidity at 8 a. m	Sample 1		Sample 2						Remarks
		Weight of seed cotton from 10 bolls	Moisture in seed cotton	Weight of seed cotton from 10 bolls	Moisture in seed	Germination		Dormant seed		
						Sixth day	Total			
	Percent	Grams	Percent	Grams	Percent	Pct	Pct	Percent		
Sept. 15	91	152.9	55.9	159.1	54.5	4	54	16	Dew.	
16	98	131.9	50.8	136.2	50.5	1	64	13	Most bolls less than ¼ inch open. Heavy dew.	
17	88	133.8	47.8	112.9	47.0	6	58	13	Most bolls ¼ inch open, a few wider.	
18	62	127.0	42.6	96.4	31.0	17	59	2	A few samples beginning to fluff. Weather dry.	
19	83	114.9	25.3	74.1	26.2	30	68	0	Nearly all bolls fluffy. No dew.	
21	91	74.0	14.9	78.9	17.7	24	72	0	All bolls fluffy. Heavy dew.	
22	80	72.5	12.8	74.2	14.6	20	83	1	Heavy fog and cloudy. Some bolls showed dew at 10.30 a. m.	
23	91	91.4	13.3	83.4	15.5	3	83	2	Heavy fog with moist atmosphere at 10.25 a. m.	
24	89	76.3	13.1	75.8	13.7	50	50	0	Weather clear with no dew.	
25	77	77.7	12.8	75.2	12.9	61	82	3	No dew.	

In 1932 the experiment was enlarged to obtain data (table 3) on several varieties, and the factor of seed dormancy was eliminated by drying and storing the seed for approximately 1 month before the germination tests were made. Three series of tests were conducted, beginning on September 7 and 20 and October 5 and covering periods of varying weather conditions. The test started on September 7 was made with the Tidewater, Cleveland, and Foster varieties. The test started on September 20 comprised these varieties and also Meade. The last test, started on October 5, was made with Tidewater and a strain of sea island.

The method of collecting the data was as follows: At the beginning of each test, approximately 250 bolls of each variety were tagged, normal bolls about one-fourth inch open at the tip being selected. On the date of tagging, 20 tagged bolls of each variety, chosen at random, were clipped from the plants. The seed cotton was removed from the bolls immediately and divided into four samples, one lock from each boll being placed in each sample. Sample 1 was ginned immediately, and the moisture content of the seed was determined; sample 2 was ginned immediately, and the seed was placed in the seed germinator to determine the percentage of germination of fresh seed; sample 3 was used to make an immediate determination of the moisture content of the seed cotton; sample 4 was sun-dried and the seed cotton was stored for 1 month before germination tests of the seed were made. This procedure was repeated each day for 10 successive days on 20 tagged bolls of each variety chosen at random, thus giving data on bolls harvested in daily lots from 1 to 10 days after opening.

TABLE 3.—*Moisture and germination percentages of seed in several varieties of cotton during the period of boll opening in 1932*

BOLLS TAGGED SEPT. 7											
Bolls harvested	Rainfall	Tidewater		Cleveland		Foster		Meade		Sea island	
		Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
	Inches	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Sept. 7	-----	52.0	91	48.7	83	50.9	95	-----	-----	-----	-----
8	-----	43.0	93	38.9	87	41.0	95	-----	-----	-----	-----
9	-----	34.7	81	29.7	88	28.5	94	-----	-----	-----	-----
10	-----	31.2	87	17.3	85	16.0	96	-----	-----	-----	-----
11	-----	13.0	96	15.9	84	12.7	96	-----	-----	-----	-----
12	-----	12.1	96	13.3	94	10.6	93	-----	-----	-----	-----
13	0.01	11.2	97	12.7	87	11.3	97	-----	-----	-----	-----
14	.80	27.2	90	27.5	97	28.3	96	-----	-----	-----	-----
15	3.61	34.0	93	34.7	87	35.9	94	-----	-----	-----	-----
16	1.74	23.3	87	22.8	81	21.6	87	-----	-----	-----	-----

BOLLS TAGGED SEPT. 20											
Sept. 20	-----	54.1	97	48.8	93	50.1	95	47.2	87	-----	-----
21	0.01	51.7	97	47.9	89	47.4	86	47.4	86	-----	-----
22	-----	45.6	89	46.3	90	36.0	100	45.5	94	-----	-----
23	.05	38.2	96	34.0	97	30.0	97	40.6	91	-----	-----
24	-----	31.0	91	30.1	93	23.3	94	39.4	94	-----	-----
25	-----	24.9	90	26.7	89	20.9	92	34.8	90	-----	-----
26	.15	27.0	81	28.7	73	23.8	79	34.5	87	-----	-----
27	.20	28.6	66	31.1	77	31.5	90	38.2	46	-----	-----
28	.35	22.5	92	27.1	76	25.2	95	34.5	62	-----	-----
29	.73	-----	78	-----	73	-----	90	-----	41	-----	-----

BOLLS TAGGED OCT. 5											
Oct. 5	-----	53.9	80	-----	-----	-----	-----	-----	-----	48.4	96
6	0.35	53.1	88	-----	-----	-----	-----	-----	-----	48.2	97
7	-----	52.2	83	-----	-----	-----	-----	-----	-----	49.0	96
8	-----	48.2	88	-----	-----	-----	-----	-----	-----	41.2	95
9	-----	33.0	92	-----	-----	-----	-----	-----	-----	29.4	96
10	-----	37.8	89	-----	-----	-----	-----	-----	-----	20.2	98
11	-----	24.5	90	-----	-----	-----	-----	-----	-----	17.1	97
12	-----	15.8	94	-----	-----	-----	-----	-----	-----	13.9	92
13	-----	11.3	97	-----	-----	-----	-----	-----	-----	12.3	92
14	-----	13.7	84	-----	-----	-----	-----	-----	-----	13.8	97

The three periods covered by the tests in 1932 afforded widely different weather conditions during the time that the bolls remained in the field. In the first test (started Sept. 7) no rain was recorded from September 7 to 12. The relative humidity for this 6-day period, as taken at 8 a. m., averaged 66 percent, and maximum temperatures of 81° F. or more were recorded each day. The seed from bolls of all varieties dried rapidly, the moisture being reduced from approximately 50 percent on September 7 to about 13 percent on September 11. Rains recorded on September 13, 14, 15, and 16 caused an immediate rise in the moisture content of the seed, but these rains, during the latter part of the test period, did practically no damage to the germinating qualities of the seed.

The second series of bolls, tagged on September 20, dried much more slowly than the first series. On September 21, 0.01 inch of rain was recorded and 0.05 inch on September 23. The average relative humidity, taken at 8 a. m., for the period September 20–25 was 85 percent, 19 points higher than the average for the first 6 days of the first test period. Because of these conditions, samples collected

on the sixth day after opening had from 20.9 to 34.8 percent moisture, whereas samples in the preceding test had had from 10.6 to 13.3 percent moisture after 6 days' exposure in the field. Rains on September 26, 27, 28, and 29 prevented the seed from further drying in the field. Germination percentages were high from samples of all varieties harvested before September 26, but lower germination percentages were obtained from nearly all samples of Tidewater, Cleveland, and Meade harvested after the rains. Seed of the Meade variety dried more slowly than that of the other varieties and showed the greatest amount of deterioration from exposure in the field. Seed of the Foster variety dried more promptly than that of the other varieties and showed practically no loss of germination as a result of the unfavorable conditions.

The third test, begun on October 5, included only the Tidewater variety and sea-island strain. On October 6, 0.35 inch of rain was recorded, and cool weather prevailed during October 6, 7, and 8, the maximum temperatures being 69°, 67°, and 74.5° F., respectively, so that drying proceeded very slowly. With higher temperatures the rate of drying increased, and by October 13 the moisture of the seed of both varieties was reduced to approximately 12 percent. Seed of the sea island dried considerably faster than that of Tidewater, the difference possibly being due to the smaller size of the sea-island bolls. Germination percentages for both varieties remained fairly constant throughout the period of the test, the sea-island seed averaging slightly higher than the Tidewater.

In 1933 further tests on the percentage of moisture and germination of seed during boll opening were made on Tidewater, Coker Wilds, Foster, and Cleveland, and on several strains of sea island. Three series of tests were made, beginning August 1, August 10, and August 28, respectively. Samples were obtained similar to those in 1932 except that the seed cotton from each variety was divided into 2 samples each day instead of 4 as in 1932. These 2 samples were used to make determinations of moisture in the seed cotton when harvested and of germination percentages of the seed after it had been dried and stored for 1 month.

The data (table 4) obtained in 1933 are in accord with those obtained in 1932. In both series, seed in all varieties contained slightly more than 50 percent moisture at the beginning of the opening period, and the rate of reduction in moisture percentages was influenced by rainfall, humidity, and temperature. In the first test, drying of the cotton was slightly delayed by rains on August 4 and 5 but progressed rapidly afterward. The viability of the seed was only slightly affected by these rains. This test was continued for only 8 days, as the bolls were all well opened and dry on August 8. The second series of bolls, tagged on August 10, dried rapidly during the first 3 days of the test, reabsorbed moisture during the rain of August 13, and dried rapidly to approximately 11 percent by August 15. Rains on August 16, 17, 18, and 19 caused reabsorption of moisture to above 30 percent. Excellent germination was obtained from all varieties of seed harvested before August 19. For all varieties, except Foster, lower germination percentages were obtained from seed from bolls harvested on August 19.

TABLE 4.—*Moisture in seed cotton and germination of seed in several varieties of cotton during the period of boll opening in 1933*

BOLLS TAGGED AUG. 1

Bolls harvested	Rainfall	Tidewater		Coker Wilds		Foster		Cleveland	
		Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
	Inches	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Aug 1	-	53.7	92	52.4	91	51.4	93	53.9	86
2	-	47.1	89	37.4	85	39.3	96	42.2	87
3	-	32.2	92	24.7	92	21.4	89	24.8	87
4	0.50	26.6	82	18.3	91	16.8	89	14.9	79
5	.44	22.0	79	18.8	85	15.9	92	16.6	89
6	-	15.6	90	14.2	93	11.3	96	13.6	88
7	-	11.3	91	9.4	89	9.2	91	8.6	74
8	-	11.4	85	9.5	92	9.4	95	9.3	78

BOLLS TAGGED AUG. 10

Aug 10	-	53.2	89	52.5	94	51.0	93	51.9	81
11	0.01	46.3	99	40.7	90	46.3	88	46.3	95
12	-	33.8	90	23.2	95	26.9	95	25.1	90
13	1.74	38.7	86	34.2	97	32.3	95	48.7	86
14	.01	14.8	94	13.2	92	16.8	96	12.6	80
15	-	11.5	92	10.7	100	12.4	95	9.9	95
16	.28	15.4	97	17.6	89	18.5	92	19.0	88
17	.14	31.7	93	31.2	95	31.2	93	39.1	86
18	.35	25.4	84	27.2	98	30.9	93	30.5	90
19	.38	31.3	67	45.4	83	37.2	92	41.4	78

¹ All moisture determinations after Aug. 13 were made on seed instead of on seed cotton.

The third series in this test was begun on August 28, data being obtained on Tidewater and on the Seabrook, Westberry, and Andrews strains of sea island. The weather during the period covered by this test was unfavorable for harvesting cotton. Rain was recorded on August 28, 30, and 31, and on September 1, 2, and 6. The data on moisture and germination percentages in this series (table 5) are of particular interest in comparing the rate of drying of the seed of the upland type, represented by Tidewater, with the seed of the sea-island strains. While the frequent rains and high humidity prevented rapid drying in any of the varieties, from the second to the sixth day of the period, the Tidewater seed contained from 15 to 20 percent more moisture than the seed of the sea-island strains harvested on the same dates.

The adverse weather conditions seriously affected the germination of the Tidewater seed harvested after the first day of the test period. Of the seed from bolls harvested on August 28, 91 percent germinated, but thereafter germination percentages were in general progressively lower as the length of exposure in the field increased. Only 13 percent of the seed from bolls harvested on September 6 germinated. Seed from the sea-island strains gave good germination percentages in practically all cases until after the heavy rains of September 6.

TABLE 5.—*Moisture and germination percentages of seed in the Tidewater variety as compared with that in several strains of sea-island cotton during the period of boll opening in 1933*

[Bolls tagged Aug. 28, 1933]

Bolls harvested	Rainfall	Sea island strains							
		Tidewater		Seabrook		Westherry		Andrews	
		Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
	Inches	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Aug 28	0 01	54 6	91	49 2	96	43 6	93	48.1	96
29	---	53 6	81	36 4	95	32 3	93	37.1	95
30	17	47 8	72	29 0	94	28 1	94	36 1	89
31	50	45 0	60	28 1	91	27 8	89	28 9	96
Sept. 1	1 15	45 9	57	32 2	96	36 5	84	37 6	92
2	01	40 5	28	21 4	90	21 4	89	19 5	93
3	---	20 2	20	14 0	91	14 5	81	13 6	82
4	---	14 6	20	12 5	64	12 0	82	11 7	91
5	---	14 9	29	13 8	89	13 6	92	13 2	93
6	10 46	55 5	13	44 0	69	52 4	50	43.0	79

The foregoing tests show that the reduction of moisture in the seed cotton and seed during the period of boll opening proceeds rapidly in periods of dry weather and high temperature. Under favorable conditions at James Island, cotton may be dry enough to pick 5 days after the bolls begin to open. In periods of rainy weather, the drying process is delayed and the moisture content of both seed cotton and seed may be considerably increased by the reabsorption of moisture. Rains which delay the drying-out process usually cause deterioration of the seed. Among the varieties tested, sea island and Foster dried more rapidly than the others and were more resistant to seed deterioration when exposed to unfavorable weather in the field.

Seeds from bolls which were just beginning to open were found to be viable and, if properly dried and stored for a short time, gave higher germination percentages than seeds which were exposed in the field for a greater length of time. From this fact it may be assumed that the picking of partially opened bolls does not lower the viability of the bulk seed of which they constitute a part, provided they are dried promptly after picking.

Owing to the high moisture content of freshly picked seed cotton in the coastal section of the Southeastern States, caused by climatic conditions, special precautions are necessary to prevent injury to the cotton. Farms usually are provided with drying platforms on which the cotton is spread and exposed to the sun for a few hours to remove the excess moisture. Drying for 1 day in bright sunshine usually is enough to reduce the moisture content sufficiently to permit safe storage or ginning. Experience has shown that seed cotton having about 10 percent moisture may be ginned safely or may be stored in bulk with little danger of heating. When more cotton is harvested than can be exposed in the available space, or when periods of cloudy weather occur, several days may elapse before it is possible to dry the cotton. Under such conditions, the damp cotton usually is stored in small piles or is spread as thinly as possible on the available floor space. Data obtained in 1932 on damp cotton stored for 6 days indicated that no deterioration in germination of the seed occurred during this short period of storage.

DETERIORATION OF SEED IN THE FIELD

It has been shown that rain occurring during the period of boll opening delays the opening process and reduces the percentage of viable seed. Further evidence of the effect of weather conditions on the viability of seed exposed in the field is shown by experiments conducted in 1931, 1932, and 1933. These experiments were planned to determine the moisture and germination percentages of seed from bolls exposed in the field for 7 and 14 days, respectively, after boll opening began.

On August 12, 1931, 200 bolls of the Tidewater variety which were just beginning to open were tagged. Rain recorded on August 12, 13, 14, and 15 totaled 2.75 inches. On August 19, 100 tagged bolls were picked for tests of moisture and germination. These bolls had been delayed in opening by the wet weather, and nearly all locks were still compact when the cotton was picked. The moisture content of the seed from this sample was 24.5 percent and the germination only 33 percent. The remaining half of these bolls were picked on August 26. As no rain had fallen between August 15 and 26, and as the period August 17-26 was one of comparatively low atmospheric humidity, these bolls were dry and fluffy. The seed contained 10.0 percent moisture and showed no further deterioration, the germination percentage being 33.

A second series of bolls was tagged on August 19 and picked on August 26. Dry weather and low humidity prevailed during this period, and the bolls were well opened and the locks fluffy on August 26. The seed from these bolls, in contrast to those opening during the preceding week, contained 9.6 percent moisture and gave a germination of 87 percent.

A group of bolls was tagged on August 26, and half of them were picked on September 2 and the remainder on September 10. Heavy rains were recorded on August 28 and 31 and on September 3 and 4, and a shower on September 5. The seed from the bolls which opened during the week of August 26 to September 2 contained 22.8 percent moisture and had a germination percentage of 48. The seed from the bolls which remained in the field for the second week and were exposed to further rains contained 12.7 percent moisture and had a germination percentage of 30. The rains of September 3, 4, and 5 had delayed the drying-out of the seed and further lowered their viability.

A group of bolls tagged on September 11 was picked on September 18 and 25. These bolls were exposed to 0.67 inch of rain on September 14, this being the only rain during the period of the test. The seed from the first series of bolls, picked on September 18, contained 12.3 percent moisture and 73 percent of them germinated. The seed from the second series, picked on September 25, contained 13.4 percent moisture and 58 percent of them germinated, indicating that the rain on September 14 caused further deterioration of the seed in the bolls left in the field. The last group of bolls was tagged on September 18 and picked on September 25 and October 2. The series picked on September 25, after being open 1 week in the field, had a germination percentage of 84 and contained 13.4 percent moisture. The series picked on October 2 had a germination percentage of 81 and contained 9.2 percent moisture. No rain fell during this 2-week period.

The germination percentages were obtained from tests made immediately after the samples were picked. Other experiments have shown that there is some degree of dormancy in freshly picked cottonseed.⁵ However, since these samples were obtained from bolls open in the field from 7 to 14 days, the number of dormant seed would be smaller than from partially opened bolls.

The data obtained in 1931 indicated that bolls opening and picked during a rain-free period would yield seed of high germination, and there was also some evidence that the seed was more likely to be damaged if rain fell shortly after the bolls began to open than if it fell after the locks had fluffed out. It has been observed that partially opened bolls are very susceptible to attack by fungus and bacterial infections and that in periods of rainy weather many partially opened bolls become so severely infected that they fail to open further during subsequent fair weather.

Further studies of cottonseed deterioration in the field were conducted in 1932. Data similar to those obtained on Tidewater cotton in 1931 were collected on Tidewater, Cleveland, Foster, and Meade (table 6). In order to eliminate dormancy as a factor, all seed samples were thoroughly dried and stored for approximately 1 month before the germination tests were made.

TABLE 6.—Moisture and germination percentages of seed from cotton exposed to varying weather conditions in the field in 1932

BOLLS PICKED 1 WEEK AFTER TAGGING

Bolls tagged	Bolls picked	Tidewater		Cleveland		Foster		Meade	
		Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Aug. 16	Aug. 23	33.8							
23	30	23.0	57						
30	Sept. 6	11.8	84						
Sept. 9	16	39.4	90	38.3	90	36.0	95		
15	22	11.0	73	11.3	84	10.5	89		
22	29	27.0	42	15.6	54	13.7	81	23.7	55
29	Oct. 6	24.1	75	17.5	83	13.1	89	31.3	51
Oct. 6	13	10.2	86						

BOLLS PICKED 2 WEEKS AFTER TAGGING

Bolls tagged	Bolls picked	Tidewater		Cleveland		Foster		Meade	
		Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Aug. 16	Aug. 30	18.4	53						
23	Sept. 6	12.0	51						
31	14	26.7	66						
Sept. 8	22	10.4	89	10.3	90	10.0	98		
15	29	12.5	47	14.5	52	12.4	77		
22	Oct. 6	10.1	33	16.9	60	17.2	77	21.8	57
29	13	9.9	87	9.7	80	9.8	91	10.1	76

As shown in table 1, the only rain-free periods of more than 4 days during these tests were August 26–September 12, inclusive, and October 7–15. The data in table 6 show that good seeds were obtained during these rain-free periods, and that germination percentages were considerably lower during periods of rainy weather. The Tidewater, Cleveland, and Meade varieties appeared equally susceptible to injury from wet weather, but the Foster variety gave a considerably higher average germination throughout the period of the test.

⁵SIMPSON, D. M. See footnote 4.

In the season of 1933, experiments on the deterioration of seed in the field were continued and the work was enlarged to provide more complete data through the picking season. Determinations of moisture and germination percentages were made on seed from the Tidewater, Coker Wilds, Foster, and Cleveland varieties. These determinations were made on seeds from open bolls exposed in the field for 7 and 14 days. The first group of bolls was tagged on August 1, and a new group in each variety was tagged each day thereafter, except Sundays, to and including August 25. Thus the daily fluctuations in moisture and germination of seed from bolls of known age were ascertained for the four varieties being tested (table 7). All moisture determinations were made on seed ginned and weighed immediately after removal from the bolls, and all germination percentages were made on seed which had been thoroughly dried and stored for approximately 1 month.

TABLE 7. —Moisture and germination percentages of seed from cotton exposed to varying weather conditions in the field in 1933

BOLLS PICKED 1 WEEK AFTER TAGGING									
Bolls tagged	Bolls picked	Tidewater		Cleveland		Foster		Coker Wilds	
		Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Aug 1	Aug 8	11.5	95	8.6	89	10.0	91	9.7	97
2	9	11.8	93	9.9	90	11.3	95	10.7	94
3	10	10.6	86	9.7	91	9.7	95	8.8	89
4	11	11.4	95	8.7	94	10.6	91	9.8	84
5	12	9.6	93	8.3	83	9.9	97	8.8	91
7	14	12.2	83	10.2	93	10.9	95	11.4	95
8	15	10.3	91	9.8	90	10.3	94	10.5	96
9	16	20.7	86	18.7	87	18.2	86	16.2	92
10	17	33.1	90	45.0	90	39.9	94	35.0	92
11	18	25.2	92	31.2	84	31.0	92	29.4	92
12	19	33.5	58	43.6	67	43.2	87	38.2	73
14	21	42.9	43	43.0	58	46.5	81	44.1	85
15	22	43.3	42	35.6	43	39.4	91	34.8	70
16	23	30.8	46	19.6	67	25.4	74	23.1	60
17	24	14.2	77	11.8	69	13.0	78	13.0	53
18	25	12.7	72	12.2	52	12.4	81	13.1	74
19	26	14.1	73	13.4	66	13.3	90	13.6	73
21	28	10.8	60	9.9	76	10.5	89	10.6	74
22	29	11.8	76	10.2	69	11.5	75	10.9	58
23	30	26.0	40	28.8	73	31.5	92	32.3	80
24	31	27.1	66	29.9	81	25.4	99	24.0	87
25	Sept 1	38.9	51	44.6	83	35.5	82	40.7	73

BOLLS PICKED 2 WEEKS AFTER TAGGING									
Bolls tagged	Bolls picked	Tidewater		Cleveland		Foster		Coker Wilds	
		Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Aug 1	Aug 15	10.0	87	9.0	86	9.1	92	9.1	96
2	16	15.9	82	15.6	87	17.5	97	17.8	93
3	17	34.4	89	34.0	75	33.0	93	32.2	84
4	18	22.9	75	21.8	78	24.1	96	22.3	89
5	19	43.4	67	42.8	73	40.2	88	35.4	75
7	21	41.2	77	36.5	80	40.3	84	39.7	75
8	22	30.2	51	28.3	76	28.2	77	27.3	61
9	23	13.4	56	10.6	79	12.3	70	11.6	62
10	24	11.3	57	10.6	85	11.0	69	11.6	70
11	25	12.8	66	13.4	63	10.5	53	13.2	61
12	26	12.0	31	11.3	57	11.4	59	11.5	48
14	28	10.4	34	9.7	45	9.9	76	10.8	44
15	29	11.7	45	10.7	43	11.4	67	11.1	42
16	30	25.1	16	29.2	33	29.6	64	31.9	37
17	31	22.6	48	26.0	49	24.9	73	26.4	47
18	Sept. 1	33.3	31	35.7	28	38.7	62	33.5	39
19	2	18.7	38	15.8	27	17.6	66	19.8	32
21	4	12.3	37	11.2	54	12.1	63	11.8	49
22	5	13.6	32	13.7	40	13.5	75	14.1	53
23	6	54.5	37	69.2	---	67.1	72	63.9	---
24	7	31.8	17	37.3	30	27.6	70	42.8	---
25	8	15.5	11	10.2	16	10.1	80	14.7	64

The weather records (table 1) for the period of this experiment show that rain fell on 20 days of the 39-day period August 1–September 8. All bolls harvested had been rained upon in the field. Although several rains were recorded, the weather during the period August 1–12 was favorable for harvesting cotton, as the rains were not excessive and the relative humidity during the day was comparatively low. Seeds of all varieties from bolls tagged before August 12 and harvested before August 19 gave high germination. Although the seed from bolls harvested on August 16 and 17 contained excessive moisture when harvested, the germination was not seriously affected. Rain was recorded on 15 days of the 23-day period August 16–September 7. Bolls opening during this period of rainy weather did not dry out promptly and the seed contained excessive moisture during practically the entire time that they were exposed in the field. Germination percentages were low in the Tidewater, Coker Wilds, and Cleveland varieties when exposed for 7 days, and in all varieties when exposed for 14 days. The Foster variety, as in 1932, showed less susceptibility to adverse conditions than the other varieties of the test, and when exposed for only 7 days produced good seed. Bolls of the Foster variety tagged on August 23, 24, and 25 remained in the field during one of the heaviest rainfalls on record in this section and still gave germination percentages of 72, 70, and 80, respectively. The seed from bolls of this variety, open for 14 days and harvested on September 6, contained 67.1 percent moisture when harvested, and yet when they were dried and stored for approximately 1 month, 72 percent germinated. This greater resistance to adverse weather conditions shown by seed of the Foster variety indicated the possibility of increasing the resistance to field deterioration in other varieties by selection.

The number of varieties upon which comparative data have been obtained is so limited that no general conclusions can be drawn as to the plant or seed characters which offer most resistance to field deterioration. However, it is probable that density of plant foliage, size of bolls, and other factors which influence the rate of drying of the seed are the more important. Among the varieties tested, all strains of sea island cotton have shown high percentages of seed germination even under adverse weather conditions. The absence of fuzz on the sea-island seed has been suggested as a possible reason for the greater resistance to field deterioration, and it may be that some advantage from more rapid drying results from this slick-seed character. However, Meade seed, which is also fairly free from fuzz, shows no advantage over that of such varieties as Tidewater, Coker Wilds, and Cleveland, which bear fuzzy seed.

SUMMARY

Seasonal fluctuations in the germination of cottonseed have been attributed to climatic conditions during the harvesting season, but the definite relationship of rainfall, humidity, and temperature to the viability of the seeds has received little attention. Experiments conducted at James Island, S. C., in 1931, 1932, and 1933, under conditions of frequent rainfall and high humidity, provide information on these conditions.

Cotton harvested at James Island normally contains excessive moisture. Determinations of the moisture content of seed during the period of boll opening showed that seed from bolls just cracking open contained approximately 50 percent moisture and that seed from partially opened bolls, which are ordinarily harvested by the pickers, may contain more than 28 percent moisture. Dry weather caused rapid reduction in the moisture content of the seed and seed cotton, but rainy, humid, or cool weather prevented drying and delayed boll opening.

The low viability of seed harvested in unfavorable weather indicates that seed deterioration occurs in the field before harvesting. Seed from bolls just opening, when dried and stored for a short time, gave higher germination percentages than seed which had been exposed for a longer period in the field.

Data on the viability of seed taken from bolls exposed for varying lengths of time in the field showed that deterioration of the seed was correlated with rains or humid conditions which prevented the prompt drying of the seed cotton after the bolls began to open. Seed from bolls opening and harvested during periods of dry weather gave higher germination percentages than did seed from bolls opening and harvested during rainy weather. Differences in resistance to field deterioration were apparent among the varieties tested, and the possibility of improvement in the germinating qualities of cottonseed by selective breeding is suggested.

RELATION OF MOISTURE CONTENT AND METHOD OF STORAGE TO DETERIORATION OF STORED COTTON-SEED¹

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INTRODUCTION

Experiments conducted at the United States Acclimatization Field Station, James Island, S. C., have shown that rapid loss of viability occurs in cottonseed stored under the humid conditions of the South Carolina coastal district. Under ordinary storage conditions at James Island seed may be safely kept for 2 years, but rapid loss in viability occurs during further storage. In the arid sections of the West, cottonseed has been kept for more than 20 years without complete loss of viability, and seed stored at Washington, D. C., has given fair germination percentages after 10 years.

DETERIORATION OF SEED IN ORDINARY STORAGE

The short life of cottonseed in ordinary storage at James Island is shown by the data (table 1) from a series of experiments begun in 1930 to determine the effect of different methods of storage on the rapidity of deterioration.

The storage house used for these experiments is a one-story frame building constructed on piers 8 feet above the ground to allow free circulation of air. The building is divided into three rooms, each approximately 10 by 15 feet, sealed overhead with matched ceiling. Each room has 2 windows, 1 on the north and 1 on the south side. These windows are equipped with glass sash and tight wooden shutters. Ventilators that may be opened or closed are provided under each window at the floor level and in the center of the ceiling of each room.

Different conditions of storage were provided in each room by controlling ventilation and sunlight; other conditions were as uniform as possible. The control of ventilation in the various rooms was as follows:

Room 1.—All shutters and ventilators open on dry days when humidity was below 80° and closed on wet days and at night.

Room 2.—All shutters and ventilators closed at all times.

Room 3.—Ceiling ventilator and window shutters open at all times. Floor ventilators open on dry days when humidity was below 80°, and closed on wet days and at night.

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² The writer wishes to acknowledge the assistance of E. H. Toole, physiologist, Division of Seed Investigations, Bureau of Plant Industry, in planning these experiments.

TABLE 1.—*Moisture and germination percentages of sea-island cottonseed stored at James Island, S. C., November 1930 to September 1933*

STORAGE ROOM 1

Date	Bags stacked on floor						Bags stacked on lattice					
	Bottom bag		Middle bag		Top bag		Bottom bag		Middle bag		Top bag	
	Mois- ture	Ger- mina- tion	Mois- ture	Ger- mina- tion	Mois- ture	Ger- mina- tion	Mois- ture	Ger- mina- tion	Mois- ture	Ger- mina- tion	Mois- ture	Ger- mina- tion
	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent
Nov 24, 1930	11 71	95	11 42	96	11 34	89	11 17	89	11 04	89	11 09	91
Mar 16, 1931	11 60	91	11 20	96	11 30	97	11 10	90	10 90	90	11 00	88
July 13, 1931	11 27	83	10 78	92	10 42	90	10 84	89	10 61	90	10 34	90
Nov 24, 1931	11 30	60	10 65	91	10 61	91	10 58	83	10 47	88	10 46	83
Mar 20, 1932	11 72	57	11 38	82	11 81	87	11 16	79	11 11	82	11 23	77
July 6, 1932	11 15	17	10 76	50	10 92	39	10 92	76	10 76	69	11 03	51
Nov 22, 1932	12 65	4	11 02	18	11 63	13	11 19	44	10 68	47	11 18	41
Mar 14, 1933	13 69	9	11 05	43	11 90	16	11 19	47	10 76	51	11 69	34
June 27, 1933	12 09	1	10 91	3	11 01	0	10 75	8	10 86	17	10 95	1
Sept. 5, 1933	12 28	0	11 32	0	11 73	0	11 11	6	11 13	5	11 91	1

STORAGE ROOM 2

Nov 24, 1930	12 47	88	13 23	92	11 95	96	10 66	89	10 86	86	11 54	87
Mar 16, 1931	12 05	95	11 10	94	11 80	93	10 80	92	10 70	91	11 30	92
July 13, 1931	11 56	88	10 79	95	10 79	90	10 69	92	10 64	93	10 54	88
Nov 24, 1931	11 73	42	10 80	84	10 74	79	10 71	82	10 75	93	10 73	85
Mar 20, 1932	12 22	45	11 84	79	11 86	64	11 62	71	11 28	77	11 72	74
July 6, 1932	11 95	9	11 10	12	11 32	28	11 27	67	10 90	69	11 26	50
Nov 22, 1932	12 57	0	11 33	12	11 78	0	11 51	25	10 79	39	11 43	20
Mar 14, 1933	12 13	0	11 65	18	12 37	1	11 21	31	10 84	16	11 90	20
June 27, 1933	11 90	0	11 15	0	11 35	0	11 01	1	11 22	10	11 45	0
Sept 5, 1933	12 22	0	11 46	0	11 58	0	11 42	1	11 16	3	11 36	0

STORAGE ROOM 3

Nov 24, 1930	11 81	95	11 41	93	11 70	94	10 86	92	11 07	95	11 26	89
Mar 16, 1931	11 30	93	11 15	98	11 35	96	11 05	87	11 00	90	11 30	96
July 13, 1931	11 01	87	10 20	84	10 16	96	10 89	91	10 54	88	10 52	82
Nov 24, 1931	10 99	84	10 31	85	10 18	87	10 67	80	10 46	86	10 49	77
Mar 20, 1932	11 17	81	10 94	84	11 27	81	11 47	79	11 20	77	11 68	72
July 6, 1932	10 82	66	10 50	74	10 67	63	10 93	56	10 68	61	10 90	60
Nov 22, 1932	10 89	38	10 77	49	11 19	40	11 22	32	10 58	26	11 21	29
Mar 14, 1933	10 98	50	11 07	54	11 66	29	10 91	44	10 64	50	11 62	28
June 27, 1933	10 75	7	10 33	7	10 42	2	10 81	7	10 81	3	10 87	0
Sept. 5, 1933	11 22	7	10 85	7	10 63	2	11 29	7	10 79	0	10 79	0

Sea island cottonseed, grown at Wadmalaw Island, S. C., in 1929, was used for these tests. The seed was stored in bins from December 1929 until the fall of 1930, when the storage tests were begun. The seed was then placed in bags containing approximately 100 pounds each, and the bags were piled in stacks, 2 bags to the tier, each stack containing 12 bags. Two stacks were placed in each room, along the north wall. One stack was started on the floor and the other was raised from the floor about 8 inches by a lattice framework. Initial moisture and germination tests were made on this seed on November 24, 1930, samples being drawn from three bags in each stack, representing the bottom, middle, and top. Subsequent determinations were made on seed from the same bags.

The average germination of the seed from the 18 bags, when placed in storage on November 24, 1930, was 91.4 percent, and the average moisture content was 11.48 percent. During the period from Novem-

ber 24 to July 13, 1931, there was little change in germination percentage in the seed from any of the bags on which tests were made, the average germination percentage for all the bags on July 13 being only 2.1 percent less than the initial germination.

The first decided drop in germination came during the period July 13–November 24, 1931. The tests made on November 24 showed decreased germination in practically all cases, but the most pronounced decreases were in seed from the bottom bags of the stacks piled on the floor in rooms 1 and 2. The seed from the stacks placed on the lattice showed only a slight decrease in germination at this date. After November 24, 1931, the percentage of germination of the seed in all storage rooms declined at an increasingly rapid rate. On March 20, 1932, when the seed was 2½ years old, the average germination of all seed in storage was 75 percent. On March 14, 1933, the average germination of all seed in storage had dropped to 31.7 percent.

The high degree of fluctuation after July 1932 in germination percentages of seed from the individual bags indicates that even the viable seeds were considerably weakened and required optimum conditions for germination. Seed stored in room 3, which received the greatest amount of sunlight and ventilation, in general gave slightly higher germination percentages during the latter part of the storage period than the seed stored in rooms 1 and 2. Seed stored in room 2, which received no sunlight or ventilation, showed the most rapid decline.

As previously noted, the seed from the bottom bags in the stacks on the floor of rooms 1 and 2 was the first to show definite decreases in germination percentages. The moisture content of the seed in these bags was slightly higher than in the middle or top bags from the same stack. In room 1, the average moisture percentage of the seed in the bottom bag from November 24, 1930, to November 24, 1931, was 11.47 percent, as compared with 11.01 percent in the middle bag and 10.92 percent in the top bag. In room 2, the average moisture content of the seed in the bottom bag was 11.95 percent as compared with 11.48 percent and 11.32 percent in the middle and top bags, respectively.

The results of the foregoing experiments show that in normal dry storage sea-island cottonseed may be preserved under the conditions prevailing in the coastal district of South Carolina for 2 years without material loss of viability. Some decrease in germination was evident during the first 6 months of the third year, but the seed retained sufficient viability for planting purposes when 2½ years old.

RELATION OF MOISTURE CONTENT TO DETERIORATION OF SEED

A series of tests was begun in July 1930 to determine the effect of moisture content on the keeping qualities of stored cottonseed. For these tests, 700 pounds of sea-island cottonseed, harvested in the fall of 1929, was thoroughly mixed, and samples taken to determine the moisture and germination percentages. Three 100-pound lots of this seed were stored as follows:

Lot 1.—Air-dried seed stored in can with perforated lid.

Lot 2.—Air-dried seed stored in can with tight lid.

Lot 3.—Air-dried seed stored in burlap bag.

At the beginning of the tests, these air-dried seed contained 10.77 percent moisture and 90 percent germinated.

The remainder of the seed was exposed to the sun for approximately 7 hours and was then divided into four 100-pound lots and stored as follows:

Lot 4.—Sun-dried seed stored in can with perforated lid.

Lot 5.—Sun-dried seed stored in can without lid.

Lot 6.—Sun-dried seed stored in can with tight lid.

Lot 7.—Sun-dried seed stored in burlap bag.

At the beginning of the tests, these sun-dried seeds contained 7.8 percent moisture and gave a germination of 85 percent. The initial determinations of moisture and germination on these lots were made in the laboratory of the Division of Seed Investigations, Bureau of Plant Industry, at Washington, D. C., from representative samples transmitted in sealed containers. All the other determinations of moisture and the germination percentages (table 2) were made at James Island. Beginning December 1, 1930, determinations of moisture and germination were made on each of the seven seed lots, usually at approximately 2-month intervals, until March 12, 1934. During the period of storage, the seed was kept in room 1 of the seed house. The cans designated as sealed were fitted with tight lids, but were not airtight, and were opened for sampling at regular intervals. All samples were drawn with a sampling tube designed to secure a composite sample from a longitudinal section through the bag or can.

TABLE 2.—Moisture and germination percentages of sea-island cottonseed, air-dried and sun-dried, stored in cans and bags at James Island, S. C., July 1930 to March 1934

Date	Stored in cans								Stored in bags							
	Lot 1 (air-dried, lid perforated)		Lot 4 (sun-dried, lid perforated)		Lot 5 (sun-dried, not sealed)		Lot 6 (sun-dried, sealed)		Lot 2 (air-dried, sealed)		Lot 7 (sun-dried)		Lot 3 (air-dried)			
	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination		
July 27, 1930.....	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct		
Dec. 1, 1930.....	10.77	90	7.80	85	7.80	85	7.80	85	10.77	90	7.80	85	10.77	90		
Jan 20, 1931.....	11.14	91	8.36	97	8.16	87	8.12	89	11.36	91	10.60	90	11.62	95		
Mar 16, 1931.....	10.90	88	8.20	94	8.07	90	7.91	94	11.22	95	10.52	93	11.52	92		
May 26, 1931.....	10.30	91	8.50	95	7.70	91	7.80	93	11.15	90	10.50	91	11.05	92		
July 20, 1931.....	10.82	92	8.17	90	7.90	95	7.86	92	10.72	86	10.09	94	10.75	95		
Sept. 21, 1931.....	10.85	81	8.41	95	7.85	91	8.01	92	10.98	87	10.41	89	10.81	95		
Nov 16, 1931.....	10.44	84	7.74	83	7.39	87	7.23	85	10.80	85	10.32	81	10.56	91		
Jan. 15, 1932.....	10.63	91	8.31	91	7.75	90	7.80	92	10.84	84	9.91	88	10.70	86		
Mar. 14, 1932.....	11.83	85	9.39	86	8.98	85	8.83	89	11.99	85	11.49	87	11.80	88		
May 9, 1932.....	11.22	79	8.98	84	8.44	87	8.44	91	11.60	84	10.99	85	11.34	88		
July 7, 1932.....	11.04	81	8.95	85	8.30	84	8.56	85	11.98	71	10.83	71	10.91	81		
Sept. 1, 1932.....	10.87	72	8.57	77	8.85	78	7.74	86	11.02	62	10.76	49	10.91	39		
Oct. 27, 1932.....	10.54	39	8.54	84	8.21	81	7.78	85	11.05	39	10.66	42	11.03	41		
Jan 4, 1933.....	11.53	48	9.04	84	9.28	86	8.37	87	11.30	39	11.17	64	11.74	45		
Feb. 27, 1933.....	11.37	44	8.94	86	9.14	87	8.40	90	11.26	32	11.20	35	11.59	31		
Apr 24, 1933.....	11.55	33	9.08	85	9.29	90	8.39	93	11.25	24	11.28	56	11.64	36		
June 20, 1933.....	11.21	34	9.15	81	9.58	89	8.20	82	11.54	19	11.24	38	11.31	17		
Aug. 9, 1933.....	11.14	28	9.09	87	9.28	80	8.12	85	11.51	16	10.99	18	11.20	11		
Oct. 3, 1933.....	10.35	0	8.96	85	8.97	79	8.21	80	11.12	1	11.07	6	11.42	1		
Dec. 4, 1933.....	11.20	0	9.27	80	9.21	77	8.20	81	11.34	0	10.89	0	11.27	0		
Jan. 8, 1934.....	11.32	0	9.30	89	9.35	81	8.31	88	11.46	0	10.81	0	11.11	3		
Mar. 12, 1934.....	11.35	0	9.23	80	9.32	78	8.28	89	11.38	0	11.05	0	11.02	0		
	11.22	0	9.20	86	9.11	78	8.23	93	11.18	0	10.58	0	10.90	0		

The data in table 2 show that the sun-dried seed stored in a burlap bag regained moisture rapidly. After approximately 4 months' storage, this seed contained 10.6 percent moisture, only 1.02 percent less than the air-dried seed stored under the same conditions. The germination and moisture percentages of the two lots stored in bags were approximately equal during the remainder of the storage period. Both lots germinated well until May 1932, after which deterioration was rapid. No appreciable advantage was gained by sun-drying and re-storing in burlap bags.

A comparison of the results obtained with air-dried seed in lots 1 and 2, stored in cans, and lot 3, stored in a burlap bag, show no appreciable difference in moisture or germination percentages throughout the tests. None of these lots of seed showed a fluctuation of more than 1.5 percent in moisture content during the period of storage, the maximum moisture content in all cases being below 12 percent.

The importance of moisture as a factor in seed deterioration is shown by the data obtained from the sun-dried seed stored in cans. Lots 4, 5, and 6, in which the moisture content was reduced to 7.80 percent before storage on July 27, 1930, retained their viability with little impairment up to March 1934. Over this period of approximately 3½ years, the moisture content of the seed in the unsealed can fluctuated between 7.8 and 9.58 percent, there being a gradual rise in moisture content as the storage period lengthened. The seed in the sealed can fluctuated between 7.8 and 8.83 percent moisture, the last determination, made on March 12, 1934, being 8.23 percent. During the latter part of the storage period, the average germination percentage of the sun-dried seed in the sealed can was slightly higher than that of the seed in the unsealed can or in the can with perforated lid.

These tests indicate that sea-island cottonseed may safely be stored for 4 or more years if the moisture content of the seed is not in excess of about 9 percent. Sun-dried seed stored in tin containers reabsorbed moisture very slowly, even though unsealed. When dried and stored in burlap bags, seed regained moisture rapidly. The type of storage did not materially affect the keeping qualities of the seed except as it prevented the reabsorption of moisture. Throughout the storage period there was no appreciable difference between the germination percentages of air-dried seed stored in a sealed can and air-dried seed stored in a burlap bag.

EFFECT OF MOISTURE CONTENT ON GERMINATION OF SEED IN DIFFERENT TYPES OF STORAGE

Further experiments on the relation of moisture content of cottonseed to deterioration in storage were begun in 1931 with seed of upland cotton. Four lots of seed, ranging from 8.75 to 13.78 percent in moisture content, were divided and stored in three ways, namely, in wooden bins, in burlap bags, and in tin containers.

Bulking the seed in piles or bins probably is the most common method of storage. The bins used in this experiment were 2 feet wide, 4 feet long, and 4 feet deep, constructed of matched lumber, and not covered.

The seed lots were obtained from a bulk lot of gin-run seed and subjected to various treatments to obtain the desired moisture content in each lot. The seed was grown in 1931, and the low initial germina-

tion was due to deterioration in the field ³ before harvesting and not to damage in storage.

Lots 1 and 2 represented air-dried and sun-dried seed and contained 9.59 and 8.75 percent moisture, respectively. Lots 3 and 4 after being wet to various degrees were passed through a drying machine to equalize the moisture and to dry them to the desired moisture content. These lots were placed in storage on November 3, 1931. Moisture and germination tests were made on each lot on November 3 and at frequent intervals thereafter until April 24, 1934.

Although the moisture content of the four lots of seed stored in bins (fig. 1, *A*) ranged from 8.75 to 13.78 percent when the seed was stored in November 1931, a difference in moisture content of only about 1 percent existed by November 1932. The dried seed, lots 1 and 2, showed no appreciable loss in vitality until June 1933, a period of 19 months. Seed wet to 11.16 percent moisture showed a decrease in germination percentage in 10 months, and seed wet to 13.78 percent showed a consistent decrease in 8 months.

The four seed lots stored in bags, being more exposed to atmospheric humidity, reached a point of uniform moisture content much more rapidly than the seed stored in bins. Although there was a difference of 5.03 percent in the moisture content of the four lots when first stored (fig. 1, *B*), after a period of 6 months the difference was less than 1 percent. While the dried seed stored in bags absorbed moisture a little more rapidly than those stored in bins, no appreciable difference in germination of these lots was shown. However, the seeds which were wet to 11.16 and 13.78 percent moisture and stored in bags dried out to a normal moisture content more rapidly than similarly treated seeds stored in bins, and remained viable for a longer period.

The importance of moisture as a factor in cottonseed deterioration is illustrated in the data from the four lots stored in tin containers (fig. 1, *C*). Like those stored in bins and in bags, these four lots contained moisture ranging from 8.75 to 13.78 percent when stored in November 1931. The tin containers, each of which held about 100 pounds of seed, were covered but not sealed airtight, and were opened for sampling at regular intervals. Some fluctuations in the moisture content of the samples drawn from the different lots were noted during the period of the test, but these were very small, seldom varying more than 1 percent from the initial moisture content.

Lot 1, representative of normal air-dried seed and containing 9.59 percent moisture at the time of storage, showed no appreciable decrease in germination until June 1933, 19 months after storage. After June 1933, germination was more variable, indicating that the seed, though still viable, was weaker and required more nearly optimum conditions to germinate. No appreciable differences were shown in the germination percentages of seed from lot 1 under the three methods of storage.

Sun-dried seed stored in a tin container at an original moisture content of 8.75 percent showed only narrow fluctuations in moisture content and germination throughout the period of storage. On April 24, 1934, approximately 2½ years after storage, the sun-dried seed

³ SIMPSON, D. M., and STONE, B. M. VIABILITY OF COTTONSEED AS AFFECTED BY FIELD CONDITIONS. *Jour. Agr. Research* 50:435-447. 1935.

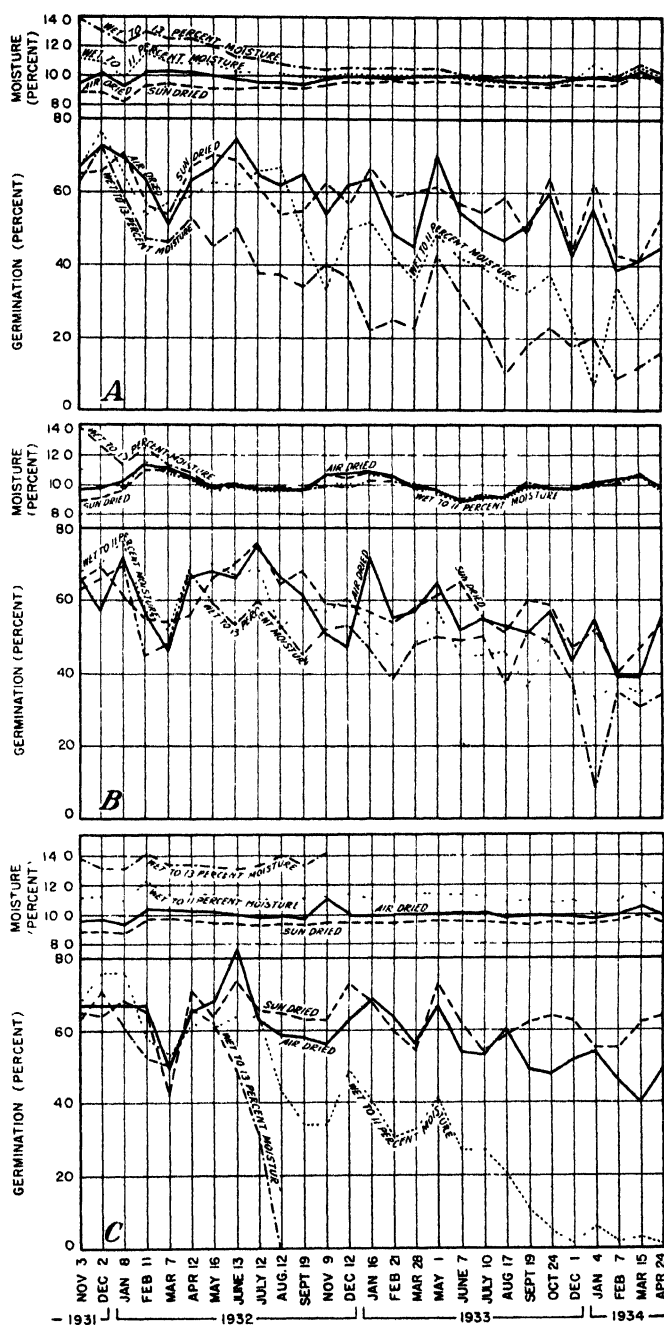


FIGURE 1.—Relation of moisture content to germination of upland cottonseed. *A*, Stored in bins; *B*, stored in bags; *C*, stored in tin containers. Solid lines indicate air-dried seed; broken lines, sun-dried; dotted lines, wet to 11 percent moisture; lines of dots and dashes, wet to 13 percent moisture.

contained 9.38 percent moisture, and 64 percent germinated. At the conclusion of the tests, this lot gave the highest germination percentage obtained from any of the stored seed.

Seed wet to 11.16 percent moisture content and stored in a tin container showed little decrease in viability during the first 8 months of storage, the test made on July 12, 1932, showing 64 percent germination as compared with an original germination of 67 percent. However, the decrease in germination was rapid after July 1932 and on April 24, 1934, at the close of the test, only 1 percent of the seed from this lot germinated.

Seed wet to 13.78 percent moisture and stored in a tin container fluctuated only slightly from this moisture percentage during the period of the test. The germination percentage of these seeds was not lowered by the high moisture content during the first 6 months of storage, but after 6 months it decreased rapidly, and the seeds were all dead 9 months after the beginning of storage.

From the foregoing tests, it is evident that a moisture content in excess of 10 percent is a critical factor in the longevity of stored cottonseed. Reducing the moisture content below 9 percent materially lengthens the time that the seed may be safely stored, provided the method of storage is such as to prevent the reabsorption of moisture from the atmosphere. If the seed contains moisture in excess of that ordinarily induced by atmospheric conditions, open storage providing ventilation reduces the moisture content and improves the storing qualities of the seed.

SUMMARY

Storage experiments with sea-island and upland cottonseed under the humid conditions prevailing at James Island, S. C., showed that in ordinary storage cottonseed deteriorates rapidly after 2 years. A definite relation is indicated between the moisture content of the seed during storage and the rapidity of deterioration. Sea-island seeds, with a moisture content reduced below 8 percent, when stored in tin containers to prevent the rapid reabsorption of moisture, retained their germination percentage with only slight impairment for 4½ years. Upland cottonseed stored under various conditions and containing from 8.75 to 13.78 percent moisture deteriorated rapidly when the moisture in the stored seed remained above 10 percent. Dried seed stored to prevent reabsorption of moisture showed only slight deterioration after 2½ years. Seed containing 13.78 percent moisture and stored to prevent drying were all dead 9 months after the beginning of storage.

A STUDY OF SOME UNPRODUCTIVE SPORTS OF THE MONTMORENCY CHERRY¹

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INTRODUCTION

The literature of fruit growing contains a number of references to barren or semibarren strains. The fact that more attention has been devoted to their occurrence in citrus than in other classes of fruits has led to the rather general assumption that they appear rarely or at least very infrequently in the common deciduous fruit groups, and that they are of relatively small economic importance. Indeed, the existence of truly unproductive variations that can be and are perpetuated as strains has been denied by some. There is, nevertheless, evidence that such strains have appeared and have been propagated, either intentionally or unintentionally, in a number of fruits. According to Carrière (2),³ such a sport of the Frankental grape was known as early as 1866, and Powell (1) described an instance of the deterioration of a commercial stock of the Fay Prolific currant through the appearance and propagation of unproductive strains. More recently attention has been called to the appearance of similar barren or semibarren strains in the Bartlett pear (8), the Downing gooseberry (12), the Montmorency cherry (5), and in several varieties of the sweet cherry (4, 6). Though there seem to be few recorded instances of the reverse type of sporting, where a barren or semibarren form gives rise to a productive type, such instances are not unknown. Thus, a double-flowered and barren form of the horsechestnut has given rise to a single-flowered branch that sets and matures seed (7), and a nonproductive variety of the Alpine currant (*Ribes alpinum sterile*) has yielded a productive form through bud mutation (3).

Apparently, in most of the unproductive strains of *Citrus*, lack of fruitfulness is associated with a failure of the blossoms to set fruit, for they are described as blossoming profusely (9, 10, 11). Most of these unproductive *Citrus* strains are said to be characterized by an exceptionally strong vegetative growth. The barren sweet cherry sports that have been described likewise blossom freely, though a large portion of their flowers are undersized and more or less defective in appearance (6), and they set little fruit. Little information is available as to the factors or characteristics that are associated with a lack of productivity in the other barren or semibarren sports that have been described.

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² Several members of the staff of the horticultural section of the Michigan Experiment Station from time to time have collected records of fruit setting or frost injury on some of the variants herein described. Thanks are especially due Dr. George F. Gray for preparing the thousands of sections of flower buds, flowers, and developing fruits that were used in this study. The photograph from which figure 2 was reproduced was supplied by R. E. Gibson, of South Haven, Mich.

³ Reference is made by number (italic) to Literature Cited, p. 478

OBJECT OF THE STUDY

Field observation in a large number of commercial Montmorency cherry orchards in Michigan during recent years, coupled with a considerable number of individual tree yield records, has convinced the writer that the appearance of unproductive bud sports⁴ is of much more common occurrence in the Montmorency variety of the sour cherry (*Prunus cerasus* L.) than has generally been suspected, and that there has been a consequent deterioration in crop yields of considerable magnitude. To ascertain the real status of the problem, a somewhat careful study was made of a number of these unproductive sports that for one reason or another had attracted attention. Descriptions of some of the more distinctive of these forms follow.

PRESENTATION OF DATA

LOW PRODUCTIVITY ASSOCIATED WITH LIMITED FLOWER-BUD FORMATION

STATION SELECTION 194

Since 1929 the Michigan Agricultural Experiment Station has had under observation, in a commercial planting of Montmorency cherries near Fennville, Mich., a large tree that has been cataloged and propagated as selection 194. When first described it had a trunk diameter of about 8 inches, being one of the largest trees in the orchard. About two-thirds of the top of this tree seems to be entirely normal for Montmorency, producing year after year medium-sized crops of normal-appearing fruit. The other third, comprising one large scaffold limb (fully 3 inches in diameter) that is more or less outgrowing the remainder of the tree, has, in each of the 5 years it has been under close observation, borne very lightly—the yield varying from an estimated one-tenth to an estimated one-quarter of a crop. The flowers on the semibarren limb appear normal in every respect and the fruits which it matures cannot be distinguished from those borne by the remainder of the tree. On the other hand, this limb bears distinctly fewer flowers, relatively, than the remainder of the tree or than is normal for trees of the Montmorency variety, and practically all its fruits are from flowers developing from buds on shoots. The spurs of this branch are practically without visible lateral flower buds, while those of the remainder of the tree each year develop a considerable, if not the usual, number of such flower buds. The failure of the spurs of this limb sport to form a normal number of lateral flower buds is well brought out in table 1 and figure 1. Over half of the spurs are entirely barren and most of those that form flower buds develop only a very small number. These wholly barren or lightly productive spurs are scattered evenly and indiscriminately over the bearing surface of the limb. The few flower buds that are produced by the spurs of the barren sport, however, are apparently as good individually as those produced elsewhere in the tree or on other trees. Why the spurs on this particular branch should fail to form flower buds freely is somewhat dif-

⁴ The term "sport" is used in this paper to refer to a whole-tree or limb variant that has been under observation for a number of years so that the permanence of its deviation from type in the orchard or in the tree is established. In most of the instances cited the variant and the accompanying parent or normal form have been propagated vegetatively. In some instances a number of the daughter trees have reached bearing age and the existence of barren or semibarren strains of bud-sport origin has been definitely established. In others the daughter trees have not been under observation long enough for a comparison to be made of their behavior with that of the parent or normal forms.

ficult to understand, for they have as many and as large leaves as the normally productive spurs. Attention is called to the fact that the so-called "normal" part of this unusual tree produces a larger percentage of barren spurs and spurs that average fewer lateral flower buds than is characteristic of other Montmorency trees of the same age in the same orchard.

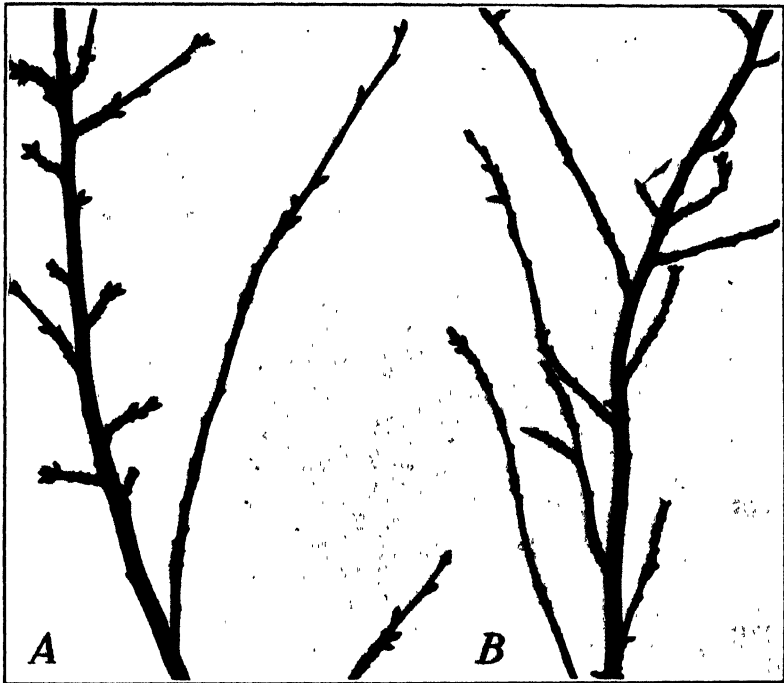


FIGURE 1. -1. A fruiting limb of the "normal" part of a tree on which selection 194 appeared as a bud sport; B, a typical limb of selection 194. Note the almost complete absence of flower buds on spurs of B.

TABLE 1.—*Flower buds on random samples of spurs from station selection 194, from the "normal" part of the same tree, and from other typical Montmorency trees, 1933*

Flower buds per spur (number)	A typical Montmorency tree (check)		Total for random samples of 190 trees in a typical Montmorency orchard		Normal part of tree having limb sport		Limb sport Selection 194	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
0.....	1	2	418	5	4	9	31	53
1.....	2	5	370	4	11	23	10	17
2.....	3	8	810	10	5	11	11	19
3.....	8	21	1,413	17	5	11	6	10
4.....	13	33	2,213	27	11	23	0	0
5.....	10	26	2,106	25	7	15	0	0
6.....	2	5	820	10	4	9	0	0
7.....	0	0	117	1	0	0	0	0
8.....	0	0	13	0	0	0	0	0
Total.....	39	-----	8,280	-----	47	-----	58	-----
Mean.....	3.7	-----	3.7	-----	3.0	-----	.9	-----

Though the lack of productivity of this bud sport (selection 194) is plainly due primarily to the partial failure of its spurs to form flower buds, its flower buds show a somewhat greater susceptibility to frost injury than those of the normal part of the tree (in the spring of 1933 the percentages killed were 34 and 17, respectively).

OTHER INSTANCES

After the discovery of the semibarren limb sport, described above, watch was kept for other sports of a similar type. Altogether, seven have been found in widely separated parts of Michigan. They differ more or less from one another, ranging from almost completely barren forms to what may be termed simply light producers. Brief descriptions of these sports are presented herewith and data on the number of fruit buds per spur are given in table 2.

TABLE 2.—Frequency distribution of numbers of flower buds per spur on certain semibarren limb sports and on the "normal"¹ portions of the same trees

	Number of spurs of selection no											
Buds per spur (number)	625, sport	626, normal	629, sport	630, normal	693, sport	694, normal	695, sport	696, normal	700, sport	701, normal	702, normal	703, sport
0	162	12	69	0	178	6	72	22	72	18	25	49
1	6	5	43	1	3	3	7	9	10	10	15	16
2	0	14	49	5	0	8	9	4	11	12	13	14
3	1	14	77	14	0	4	4	14	15	9	11	10
4	0	15	74	31	0	16	3	13	7	20	5	0
5	0	11	79	35	0	13	0	4	5	20	4	0
6	0	6	25	17	0	7	0	0	0	9	0	0
7	0	3	2	0	0	1	0	0	0	1	0	0
Total	171	80	418	103	181	58	95	66	120	99	73	89
Mean	.05	3.1	2.9	4.4	.02	3.6	.5	2.0	1.1	3.0	1.6	.8

¹ What is here designated as "normal" is, more accurately, the nonsporting portion of the tree. In reality it is in some instances, something intermediate between the true normal condition for the variety and that found on the sporting limb.

Selection 693 is a limb sport discovered in 1933 in the orchard of W. McManeth, north of Traverse City. Like selection 194, it is a large scaffold limb in a tree that is 7 to 8 inches in diameter. It is, however, almost completely barren, for flower buds are formed on less than 2 percent of its spurs, though all of them have the usual amount of foliage. The few blossoms and fruits that it bears seem to be entirely normal for the Montmorency variety.

Selection 625, another limb sport, was found in 1933 in the orchard of J. Flack in the Grand Traverse district. It resembles selection 693 more closely than it does selection 194 in that almost all of its spurs are barren. This branch is about 2 inches in diameter and originated as a limb sport in the top of a tree that must have been at least 8 or 10 years old at the time the sport appeared. Its position in the tree is such as to lead to the belief that the terminal bud on an oblique upright branch suddenly sported in this way, for the lateral branches on this branch up to a certain point are all normal in spur growth and productivity, while those that originate above that point bear spurs of the type just described. The limb sports 194 and 693, on the other hand, developed from lateral buds.

The limb sport cataloged as selection 700, discovered in an old tree in the orchard of D. H. Metzger, Traverse City, in 1933, closely resembles no. 194, in that it constitutes a main scaffold limb (4 to 5 inches in diameter, in this instance) and that it is a semibarren form rather than an almost completely barren form like nos. 625 and 693. Two-thirds of its spurs are without flower buds, and the remainder form a smaller number than do the spurs on the main, normal part of the tree (table 2). The normal part of the tree is very productive. Counts made shortly after a period of severe cold in February 1934, when temperatures as low as -30° F. were recorded in the neighborhood of this orchard, showed that 49 percent of the flower buds of this limb sport had been killed, whereas only 12 percent of those on the normally productive part of the tree (no. 701) had been killed. Similar differences were not found between the other barren or semibarren limb sports described in this group and their normal checks. The low productivity of this particular limb sport is, therefore, to be attributed at least in part to the susceptibility of its flower buds to winter injury.

The limb sport cataloged as selection 629 was first noticed in 1930. It was found in the very top of a tree, then about 25 years old, in the experiment station orchard at East Lansing, the branch in question being about one-half inch in diameter. The tree as a whole has been normally productive, but each year this branch produces a very light crop. Reference to table 2 shows that, though its spurs averaged nearly 3 flower buds each, this is only about two-thirds the number produced by the spurs on the normal part of the tree. Thirty-eight percent of its spurs had less than 3 buds each, while only about 6 percent of the spurs of selection 630 had less than 3 each. The semibarren condition of this particular limb sport is due in part to the fact that a much lower percentage of its blossoms set fruit than do those on the main part of the tree. Further reference to this characteristic will be made later.

The tree in which the forms cataloged as selections 702 and 703 were found is located in the orchard of John Stanek, at Traverse City. One part of this tree, the main portion (selection 702), is moderately productive and matures its fruit at the usual ripening season for Montmorency. Its fruits appear normal in every respect. Reference to table 2 shows, however, that its fruit spurs averaged only 1.6 flower buds each—half the number characteristic of Montmorency. Especially significant is the fact that a third of the spurs were entirely without flower buds. Though this tree is perhaps a little less favorably located with respect to soil than most of the others in the same orchard, the trees immediately surrounding it had spurs with the usual number of flower buds. All have had uniformly good care, and the small number of buds per spur cannot be attributed to lack of soil fertility or poor foliage. The evidence indicates that what is here called the "normal" part of the tree is intermediate between a fully productive condition and low productivity.

A limb sport in this same tree, cataloged as selection 703, is light-yielding and matures its fruit from 10 days to 2 weeks later than normal. Over half of its spurs are barren, while the remainder have only a few flower buds (table 2). The barren spurs on both parts of this tree—i. e., on the parts cataloged as selections 702 and 703—are

distributed more or less evenly or indiscriminately over the entire bearing surface.

A tree located in the orchard of J. Flack on the Old Mission Peninsula north of Traverse City, is interesting in that it apparently represents a condition intermediate between the one furnishing selections 702 and 703 and that furnishing selections 625 and 626. In this instance the tree is relatively young (set in 1925) and very vigorous. A third of the spurs on its "normal" part (cataloged as selection 696), however, in spite of its excellent care, vigorous condition, healthy foliage, and the barely moderate crop that it bore in 1933, formed no flower buds for 1934, though the spurs that did form buds developed a normal number. Three-fourths of the spurs on its large, semibarren limb sport (cataloged as selection 695) failed to form flower buds, and those that did develop them differentiated only a little more than half the number characteristic of the variety (table 2). Thus, the moderately productive main part of the tree (no. 696) is comparable to selection 702, while its semibarren limb sport (no. 695) is intermediate in type between selections 703 and 693. The distribution of the barren spurs on the smaller limbs of both no. 696 and its limb sport, no. 695, roughly resembled that of color striping in certain sectorial chimeras.

Another limb sport of this same type, i. e., one whose fruit spurs were barren or nearly so, was discovered in 1931 in the orchard of George Wheeler, near Shelby. It was cataloged as selection 597 and buds were obtained for propagation, but exact counts were not made of the number of flower buds per spur.

It is evident from the foregoing descriptions that these Montmorency sports, characterized by fruit spurs that develop few or no flower buds, constitute a series. Some like no. 693, are almost completely barren; others, like nos. 696 and 626, fall only a little short of being moderately productive. Between the two extremes may be found almost any intermediate form.

BARRENNESS ASSOCIATED WITH DEVELOPMENT OF LEAF BUDS IN PLACE OF FLOWER BUDS

In 1932 Drain (5) described a barren limb sport of the Montmorency, then being propagated at the Michigan station as selection 152 (fig. 2), in the following terms:

This is a sector of an old Montmorency tree growing in the McCrasslin orchard of the late Amos Tucker, of Bravo, Mich., and has been observed for a number of years. The off-type sector has outgrown the rest of the tree. Its foliage is dark green in color and very dense, except on young, rapidly growing shoots. Spurs are produced in profusion, but very few blossoms. * * * The few fruits produced on the vegetative portion of the tree appear normal for Montmorency.

Closer examination of the parent tree shows that the normal portion possesses the spur-bearing habit in a marked degree—i. e., very few of the lateral buds on the shoots, even the very short shoots differentiate flower parts—and consequently practically the entire crop is spur-borne. Similarly, the lateral buds on the shoots of the barren limb sport are exclusively, or almost exclusively, leaf buds. On the other hand, while the spurs on the normal portion of the tree develop the usual number of flower buds and regularly set and mature a large crop of fruit, the buds that develop in the axils of the spur leaves on the barren limb sport are leaf buds.

Sections made through these buds collected at successive stages throughout the summer, fall, and winter show them to be practically identical in structure with the terminal leaf buds on the same spurs or the terminal buds of the spurs on the normal part of the tree. These lateral leaf buds are smaller and more pointed than the flower buds occupying a similar position on the spurs of the normal part of



FIGURE 2.—Tree in which selection 152 originated as a limb sport (limb in foreground, lettered *BM*). Note its tendency to outgrow the remainder of the tree and the density of its foliage, as compared with that on the normal limb at the left.

the tree (fig. 3). In the spring when growth starts, these leaf buds open, and each one produces a small rosette of leaves, just like the normal terminal bud of the spur, though, owing to competition and the consequent crowding, a considerable percentage of the growing points die and are abscised. Nevertheless, some of them are able to survive, the result being the gradual development of much-branched

spurs. This gives the older portions of the tree the appearance of possessing a very compact, dense type of growth (fig. 2). Occasionally, one of the lateral buds on the spurs of this limb sport matures fruit, but the percentage of such buds is less than 1. Sections of buds from this limb sport collected at intervals throughout the year show that in those few instances where flower buds are formed, differentiation takes place at the usual season, flower development is normal, and the flowers open when the rest of the tree is in blossom. Trees



FIG. 3.—Representative spurs of selection 152 are shown in *A* and from the normal portion of the same tree at *B*. Note the difference in the size and shape of their lateral buds. The lateral buds of the limb sport no. 152 do not differentiate flower parts.

of bearing age propagated from this limb sport reproduce its characteristics faithfully.

In the summer of 1933 two other limb sports, almost identical in appearance with selection 152, were found in orchards of different ages in the vicinity of Traverse City, and two others were found in an orchard about 10 years old near Eau Claire. The Traverse City sports are now being propagated as selections 615 and 627, and the Eau Claire variants as selections 656 and 658. In the Eau Claire orchard yielding selections 656 and 658 there is a whole-tree variant of the same character (cataloged as selection 654), which has in turn produced sev-

eral small branches whose spurs show a complete return to the normally productive condition.

Still another limb sport of this same type (cataloged and propagated as selection 608) should be recorded in this connection. To the casual observer it appears practically identical with nos. 152, 615, 627, etc. It differs, however, in that though some of its few flower buds open at the same time as those on the normal part of the tree (selection 609), others are only half or a quarter grown, and their blossoming season is prolonged for a full month beyond that which characterizes the parent variety. Numerous sections of lateral spur buds of this limb sport collected at regular intervals indicate that, though

very few ever differentiate flower primordia, and though a considerable percentage of the few that do, differentiate them at the usual time, in some buds differentiation occurs a month or 6 weeks later than normal. This is well brought out in figure 4. In this particular sport, it is the buds whose flower primordia differentiation is "post-season" that develop slowly and open late the following spring.

LIGHT PRODUCTION ASSOCIATED WITH SUSCEPTIBILITY OF FLOWER BUDS TO KILLING BY LOW MIDWINTER TEMPERATURES

Winter-killing of flower buds has always been recognized as more or less of a limiting factor in sour cherry production, as it is with other

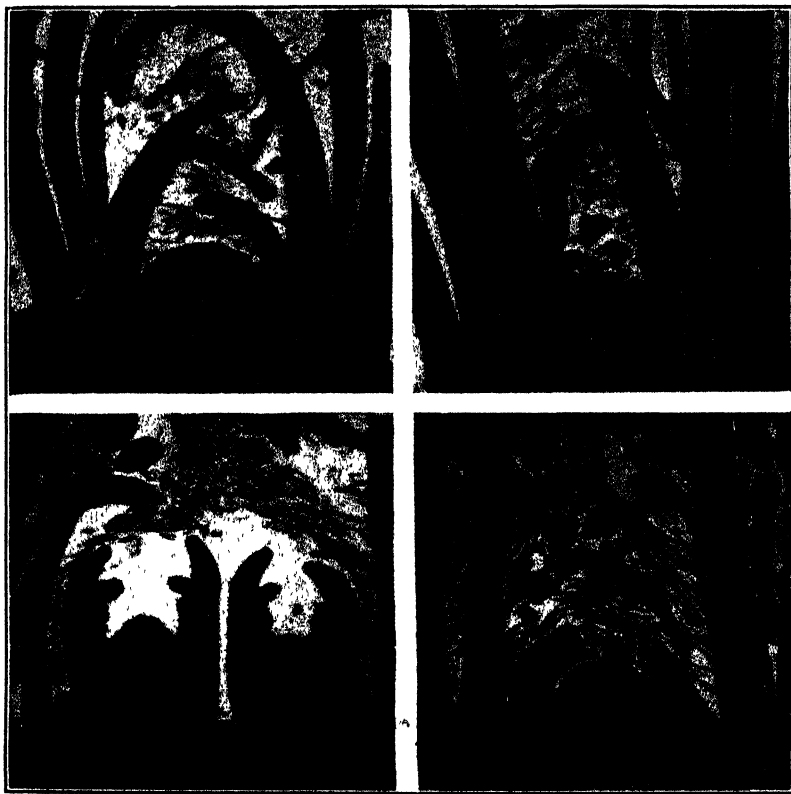


FIGURE 4 —A and B: Sections through the growing points of representative lateral spur buds of a normal Montmorency cherry tree collected July 14 and August 14, respectively, showing early and well-advanced stages of flower-bud differentiation; C and D, corresponding sections through the growing points of the low lateral spur buds on its semi-barren limb sport (selection 608) that differentiate flower parts. By August 14 the flower buds reach about the same stage of development attained by those of its parent tree a month earlier ($\times 65$).

deciduous fruits, though observation indicates that it is relatively of much less importance than the killing of opening buds by spring frost. In the effort to ascertain the causes of the light cropping of many cherry trees it was decided to look for evidence of bud sports that might be susceptible to low winter temperature. The data on several of the more striking cases follow.

SELECTIONS 262 AND 263

In the spring of 1932 an old Montmorency tree was found in the orchard of Clayton Riley, near Mears, which had one limb, about 2 inches in diameter, carrying only a third or half a crop of blossoms, while the remainder of the tree was carrying a normal crop. Many of the flower buds on the light-blossoming limb had not opened and many of those that had opened were producing only 1 or 2, instead of 3 or 4, blossoms. Examination showed that the individual flower rudiments in these buds were dead, presumably from midwinter freezing. The light-blossoming limb in this tree was cataloged as selection 262, and the normal, main part of the tree as selection 263. Limb 262 matured a very light crop in 1932, while the rest of the tree produced a heavy crop. The winter of 1932-33 was one of ordinary temperatures and there was practically no winter-killing of flower buds on either part of this tree. However, there was considerable frost injury in the spring of 1933. Count of a random sample of blossoms showed that 48 percent of those on limb sport 262 had been killed, and 53 percent of those on selection 263. Both parts matured a medium-sized crop of fruit in the summer of 1933. In February 1934, there were unusually low temperatures. Examination of flower buds collected March 10 showed winter-killing of 17 percent of the individual flowers in selection 263 and 47 percent of those in its limb sport (no. 262). In July 1934, limb sport 262 matured a medium-sized crop, while the normal part of the tree matured a heavy crop—and this in spite of the fact that counts made at the blossoming and harvesting seasons showed that fruit setting was substantially higher on the limb sport than on the normal part of the tree.

SELECTIONS 264 AND 265

About 200 yards from the tree in which selections 262 and 263 were found is another, several years younger, that in 1932 similarly showed rather marked differences from limb to limb in the percentages of flower buds that had been winter-killed, followed by corresponding differences in yield. The limb showing the largest amount of winter-killing of flower buds and lighter yield was cataloged as selection 264; the remainder of the tree as selection 265. As in selections 262 and 263, there was no killing of flower buds during the winter of 1932-33. Both parts of the tree showed the same amount of frost injury to blossoms (28 percent) in the spring of 1933, and both parts matured a good crop the following July. Following the low temperatures of February 1934, examination showed that 75 percent of the blossom buds on limb no. 264 were killed, while only 53 percent of those on no. 265 were killed. This at first does not seem to be a great difference, but when translated into terms of survival, it is a difference of 2 to 1 and corresponds rather closely with the subsequent (1934) yields of the 2 portions of the tree.

SELECTIONS 481, 482, AND 482A

A tree of the Montmorency variety with a limb sport of peculiar growth habit, producing small-sized, early maturing fruits, was found in an old orchard belonging to George Andrews, near Shelby. It has been under observation since 1929. The east side of this tree

(cataloged and propagated as selection 481), which constitutes about a third of the entire top, in vegetative, flower, and fruit characters appears entirely normal for Montmorency. The remainder of the tree (propagated as selection 482) is somewhat more vigorous and upright in growth, with thicker shoots. Its internode length averages about $1\frac{1}{2}$ inches, as compared with 2 inches for no. 481; its flower clusters average 2.4 flowers each, as compared with 3 for no. 481; its fruits in 1933 averaged 2.68 g in weight as compared with 3.44 g for no. 481. They mature about 10 days earlier than the normal Montmorency cherries and are somewhat milder in flavor.

In some seasons both parts of this tree have been equally productive; in others the aberrant sector, no. 482, produced a relatively lighter crop than the normal sector, no. 481. In the spring of 1933 it was noted that not only did many of the flower buds on sector 482 fail to open, but on 1 of its branches (about $1\frac{1}{2}$ inches in diameter) very few were starting to open, while practically all were opening on the normal sector, no. 481. Examination showed that the buds failing to open had been winter-killed. Here then, were three parts of a tree presenting markedly different amounts of winter-killing of flower buds. The limb showing the most serious winter-killing and growing out from sector 482, was propagated as selection 482A. Some frost injury occurred in this orchard a week or 10 days before the full-bloom stage, and when the blossom buds were in that stage of development, those on all three parts of the tree suffered about the same amount of injury, 25 to 30 percent being killed. Furthermore, approximately the same percentage of the blossoms that survived winter and frost injury on the three parts of the tree set and matured fruit. The crops borne corresponded closely to the amounts of winter-killing of the flower buds—being heavy, moderate to light, and very light on sector 481, 482, and 482A, respectively.

Following the low temperatures of February 1934, counts were made of large random samples of the flower buds from the three sectors of this tree. The percentages killed were 12.5, 42.5, and 67.7 on parts 481, 482, and 482A, respectively. The killing on sector 481 was not severe enough to reduce the crop appreciably; indeed, that sector actually matured a very heavy crop. Killing on sector 482 was enough to reduce the crop considerably, and that on limb 482A was enough to reduce it drastically. The 1934 yields actually produced were in line with what one would expect from the amounts of winter-killing of flower buds.

SELECTION 159

The whole-tree variant catalog as selection 159 is one of a large block of trees planted in 1905 near Sodus, Mich. It is thrifty, vigorous, and large enough to average 150 to 200 pounds of fruit per year. In no season during the 5 years that it has been under the author's observation has it borne more than 25 pounds of fruit, though trees surrounding it have yielded heavily. The owner states that it has always been either light-cropping or semibarren.

In March 1933, examination of flower buds from this tree showed that a large percentage were dead (exact number not determined), though there had been practically no killing of those on surrounding trees. This, coupled with the fact that examination in early November 1932 showed no dead flower buds and that the winter of 1932-33

had been comparatively mild, led to the belief that flower buds of this tree are especially susceptible to injury from winter cold. There was some spring frost injury in this orchard in 1933, averaging 30 percent on the trees surrounding selection 159 and 71 percent on selection 159. Following the severe winter of 1933-34 this tree showed 59 percent winter-killing of flower buds, while surrounding



FIGURE 5.--A small reverting limb that is normally productive growing out as a lateral from a larger branch in semibarren selection 159.

trees averaged only 21 percent. There was no spring frost injury in this orchard in 1934.

It is evident from these data that the flower buds of selection 159 are markedly more susceptible to injury from cold, both in midwinter when they are dormant and in late April when they are about ready to open and frost becomes a limiting factor, than are most Montmorency cherry buds. Without question, the low productivity of this tree is in considerable part due to the tenderness of its flower buds, though it is likewise due in part to the poor setting of its blos-

soms, a matter that will be discussed later. It is significant that two small branches, perhaps half an inch in diameter, near the top of this old tree have shown a full return to normal productivity (figure 5). No records have been obtained on blossom-bud killing or fruit setting on these branches, but presumably they are as hardy and the flowers set fruit as well as those on other Montmorency trees in this orchard.

SELECTION 700

As stated earlier (p. 461), the limb sport cataloged as no. 700 showed winter-killing of flower buds amounting to 49 percent following the severe winter of 1933-34, while the buds on the normal part of the tree (no. 701) showed only 12 percent killing. Here, again, susceptibility to injury from cold furnishes only part of the explanation of the near-barren condition of the sport. It is, nevertheless, a factor of considerable importance.

LOW YIELDS DUE TO DELAYED WINTER-KILLING OF FLOWER BUDS

In some instances the flower buds of the Montmorency cherry may be very resistant to low temperatures throughout the winter while they are in a dormant condition, only to become very sensitive as growth is resumed in early spring. This, however, is associated with very early spring freezing and probably is to be regarded as a delayed form of winter-killing rather than killing from spring frosts which come later.

SELECTIONS 698 AND 699

In the summer of 1933 a limb sport was found in an old Montmorency tree in the orchard of D. H. Metzger, at Traverse City, which was nearly barren. The main part of the tree was normally productive and carried a heavy crop. The twig growth, shoots, spurs, foliage, and fruits of the nearly barren branch appeared to be entirely normal, but the crop was less than a tenth of what it should have been. Later examination showed that the spurs on this part of the tree produced the usual number of flower buds, and sections of these flower buds revealed no peculiarities of structure or development. Samples collected from both the sporting branch and the normally productive part of the tree on March 23, 1934, while they were still in a dormant condition, showed that 21 percent of the individual flower buds of the nearly barren limb sport had been killed by the unusually low temperatures of the preceding winter, while only 5 percent of those on the productive part of the tree had been killed. This was a significant difference but not enough to account for the difference in productivity of the two parts of the tree. However, when a check was made on May 14, 1934, several days before the full-bloom stage, it was found that in 80 percent of the flower buds of the semibarren limb sport all of the individual flower buds had been killed, while the corresponding percentage for the normally productive part of the tree was 11. Furthermore, those buds on the limb sport from which flowers had emerged were producing only 1 or at most 2 each, instead of the usual 2 to 4. Total delayed winter-killing of individual flowers on this limb sport, therefore, amounted to at least 90 percent of those that had survived midwinter killing, whereas not more than 20 percent had succumbed on the main, normal part of the tree. However, as the season advanced, the developing

flower buds of this limb sport showed a resistance to low temperatures equal to that of the normal part of the tree, for a light frost on the morning of May 12 destroyed a slightly smaller percentage of the buds on this limb than on selection 699 or on surrounding trees. Examination showed that the buds on this semibarren limb sport paralleled in their differentiation and development those on its normal parent tree, their tender early spring condition not being due to greater or lesser advancement.

SELECTIONS 680 AND 681

A limb sport designated as selection 680, discovered in another orchard in the Traverse City section in 1933, closely resembles the semibarren no. 698, except that its blossoming season is about a week later and its maturing season about 2 weeks later than its parent form (No. 681). Examination of large random samples of its flower buds on March 23, 1934, failed to reveal a single dead one on the normally productive part of the parent tree. However, 76 percent of the flower buds of the semibarren limb sport succumbed to the delayed winter-killing that occurred later, while the corresponding percentage for the productive portion of the tree was 23. In this instance the owner of the orchard had observed this tree for many years and stated that the limb sport had never borne a full crop, while the remainder of the tree had been consistently productive.

SELECTION 697

A whole-tree variant of the same type as limb sports 680 and 698 was found in still another orchard near Traverse City in 1933. It was healthy, vigorous, well cared for, and of a size to produce a hundred-pound crop in 1933. Actually, it yielded about 3 pounds. It differentiates the usual number of flower buds, and examination revealed no peculiarities in their structure. Ninety-five percent of its individual flower buds survived the severe winter of 1933-34 (examination of Mar. 23, 1934)—about the same percentage that survived on surrounding normally productive trees. A check made May 14, however, showed that in 71 percent of its flower buds all of the individual flowers had been killed before opening and apparently an equal percentage in the remainder that did open. The presumption is that this tree was accidentally propagated from a limb sport similar to selection 698.

LOW YIELDS DUE TO SUSCEPTIBILITY OF BUDS OR FLOWERS TO FROST

In the discussion of selection 159 (p. 457), attention was called to the fact that not only are the dormant flower buds of this tree markedly susceptible to low temperatures, but its opening flower buds are likewise more susceptible to frost injury than those of surrounding normal trees. The percentage of flower buds on this tree killed by frost in the spring of 1933 was 71, while the corresponding figure for a composite sample from the surrounding trees was 30.

In early June 1932 the attention of the writer was called to two adjacent Montmorency cherry trees in an orchard of several hundred trees of that variety on the farm of Abel Teichman, at Eau Claire. Each of the two trees had a height of about 17 feet and an equal or slightly greater spread. Destructive frosts had occurred in late April,

and there had been considerable frost injury in this particular orchard. Examination showed clearly that both of the two trees had blossomed heavily, but that one had suffered only slight injury to its blossoms while the other had suffered extensively. At harvest 185½ pounds of fruit was picked from one tree (designated as selection 511), while the other (selection 512) yielded only 7¼ pounds. Both trees made a reasonable amount of new growth in 1932, though selection 512



FIGURE 6.—A, C, and E. Photomicrographs showing the November 9, April 5, and April 29 stages of representative flower buds of selection 511, a form whose blossom buds are resistant to spring frost injury; B, D, and F photomicrographs showing corresponding stages of selection 512, an adjoining tree whose blossom buds are very susceptible to spring frost injury. Both of the pistils in F and one of those in E were killed by a frost that occurred 3 days before the flowers were collected for sectioning. A, B, C, and D $\times 33$; E and F $\times 7$.

came through the season in better condition and with a much larger number of fruit buds because of the light crop that it carried. On April 25 and 26, 1933, low temperatures again occurred in southwestern Michigan, and there was more or less frost injury to opening flower buds in the Eau Claire district.

Counts made April 29 showed that 87 percent of the individual flower buds on tree 512 had been killed, while only 27 percent had been

killed on tree 511. The frost-resistant tree yielded 142 pounds of fruit in 1933, while the frost-susceptible tree yielded only 77 pounds. This difference in yield was much smaller than would be expected from the difference in frost injury to the blossom buds, principally because of the poorer budding (i. e., the development of smaller numbers of flower buds) of the frost-resistant tree occasioned by its heavy cropping in 1932. Sections of random samples of the flower buds from these two trees collected in early November 1932, March 30, 1933, and of pistils collected at the time of the frost (fig. 6) showed no differences in size at any stage or in degree or rate of differentiation of flower parts. The flowers of both were at apparently identical stages of development when the injurious frosts occurred, thus indicating that the variations in hardiness could not be associated with anatomical differences.

Two other whole-tree variants characterized by extreme susceptibility of their flower buds to spring frost may be mentioned here. Both are in the Crowley orchard about 15 miles north of Traverse City. Though the site of the orchard is one where considerable frost injury is to be expected, the orchard as a whole has been moderately productive, while these two trees have usually borne light crops. Flower buds are formed freely by both trees, and the fact that they suffered little injury from temperatures ranging from -20° to -25° F. in February 1934, indicates that they are resistant to cold while in a dormant condition. However, after frosts occurring from May 10 to 14, not a single live flower bud could be found on tree 689, while the percentage of flower-killing of a large composite sample from the four surrounding trees was 10. The other tree, no. 690, had 94 percent of its flower buds killed, while the four bordering trees had 11. Obviously, these are instances of extreme susceptibility to frost. The presumption is that all of these whole-tree variants are in reality bud sports, having been propagated from unrecognized limb variants of this kind. That such variants actually occur as limb sports is illustrated by station selection 602, a light-yielding limb in an 8- to 10-year-old tree in the orchard of D. H. Metzger, near Traverse City. This tree has been under the author's observation for several years. Records show that its low productivity (ranging from about a third to a half that of the normal part of the tree) is not due either to a noticeable susceptibility of its dormant flower buds to low midwinter temperatures or of its swelling flower buds to the delayed or late type of winter-killing. On the other hand, its flower buds are relatively very susceptible to killing by late spring frosts. Thus, a series of frosts occurring between May 10 and May 14 in 1934, which killed 27 percent of the blossom buds on the normal part of the tree (selection 601) and about the same percentage on surrounding trees, destroyed 72 percent of the flower buds on this limb sport.

LOW YIELDS DUE TO POOR SETTING OF FRUIT

Earlier in the paper (p. 457) attention was called to the fact that in the case of some of the barren or nearly barren citrus fruits and sweet cherries that have been described, the failure to produce fruit was associated either with anatomically defective flowers or with the inability of apparently normal flowers to set fruit. A number of barren and light-producing forms of the Montmorency cherry due to the latter factor have been encountered in this study.

SELECTION 676

In the summer of 1933 in the orchard of F. E. Broesch at Traverse City a tree was found about two-thirds of which was bearing a normal crop of fruit. One large limb, in vegetative characters indistinguishable from the rest of the tree, was almost barren, and the few fruits that it produced were of only about two-thirds the usual Montmorency size. The flower buds on this limb (selection 676) were examined from time to time and found to be differentiating and developing their parts in the usual manner. In the spring of 1934 this limb produced a very heavy crop of blossoms, which, however, were only about two-thirds as large as those on the normal part of the tree. Otherwise, they appeared entirely normal. There was no frost injury to the blossoms on this tree in 1934. Fruit setting on this limb, as determined from a large random sample of flowers, amounted to only 0.7 percent, while that on the remainder of the tree was 12 percent. At harvest time in 1934 the tree presented essentially the same picture that it had in 1933.

SELECTIONS 634, 635, AND 636

In June 1933 in one of the so-called "Corporation" orchards at South Haven, Mich., a tree was noted in which one sector was bearing a heavy crop, another sector a very light crop, and a third sector was completely barren. The first sector, which constituted perhaps a third of the top, yielded 16¼ pounds of fruit; the second sector, which constituted nearly two-thirds of the top, yielded 2¼ pounds; the barren part consisted of a single limb only about 1 inch in diameter. These sectors were cataloged as selections 634, 635, and 636, respectively. Periodical examinations between the harvest season of 1933 and blossoming in 1934 showed that flower-bud differentiation proceeded in a normal manner in all three parts of the tree. There was practically no winter-killing of flower buds on any of the branches, and there was no spring frost injury to the blossoms. All three parts blossomed heavily in 1934, and the blossoms appeared entirely normal. Fruit setting on the three sectors, as determined from large random samples of the blossoms (864, 606, and 1,348), amounted to 42, 33, and 0.5 percent, respectively. Sector 634 yielded 56 pounds, sector 635 yielded 20 pounds, and sector 636 yielded only a few fruits. Here is an instance of three distinct conditions of fruitfulness in a single tree, occasioned by differences in the ability of the flowers to set fruit, comparable in a way to the tree yielding selections 481, 482, and 482A, which presents three different conditions of hardiness of flower buds to winter cold.

SELECTION 160A

In the spring of 1923 three farmers near Eau Claire, (A. H., Albert, and William Prillwitz) purchased stock for commercial plantings of the Montmorency cherry from a single nursery. When the trees reached bearing age, 2 out of a total of 110 in one orchard proved to be barren, 7 in another, and 20 in the third. The other trees in the three orchards have been normally productive. Though most of these barren trees have been cut down within the past 2 years, several have been retained as curiosities. Field observations indicate that all 29 barren trees were of the same type (designated as selection 160A)—vigorous, thrifty growers, producing round or roundish upright tops

and characterized by rather coarse, stiff, thick shoots and exceptionally large flower buds. The trees bloom profusely each spring. Sections of flower buds collected in late fall and again in early spring, and sections of the pistils just before the opening of the flowers and again at full bloom indicate an entirely normal procedure in the differentiation and development of the several parts of the flower. At blossoming time the trees give every promise of maturing a full crop. The blossoms, however, fail to set fruit, practically all of them having abscised within a week or 10 days after petal fall. Fruit setting, as determined from counts of large random samples of blossoms, has in each of the past 3 years fallen short of 1 percent. Each of these trees may mature 2, 3, or even 100 fruits, which appear to be typical Montmorencies, but the trees may be considered as practically barren.

SELECTION 365

For 6 or 8 years 1 Montmorency cherry tree growing in one of the Corporation orchards at South Haven, has attracted attention because of its late-blossoming and late-maturing habit. It reaches the full-bloom stage from 3 days to a week later than surrounding trees and it matures its fruit on an average about 2 weeks later than normal. It has never yielded a satisfactory crop for a tree of its size, though in 1930, when there was more or less of a crop failure in the whole orchard on account of spring frost injury, it produced 11¼ pounds, as compared with an average of 7½ pounds for the entire block of 190 trees. In 1931, when the average yield in the orchard was 66 pounds to the tree, this particular tree bore no fruit (due largely to frost injury). In 1932 it produced 21 pounds, as compared with an average for the orchard of 57; in 1933 it bore just 18 cherries, as compared with an average of 22 pounds; and in 1934 it bore 14 pounds, as compared with an average of 109 pounds. Each year it has blossomed profusely, so that yields of at least 50 pounds might reasonably be expected. Records of fruit setting were obtained for each of the 190 trees in this particular block in 1932, 1933, and 1934. For this tree the percentages of the blossoms setting fruit in the 3 years, were 1, practically 0, and slightly less than 2, while the averages for the entire orchard were 10, 13, and 24. Obviously, the tree is not barren to the same extreme degree as limb sports 676, 636, or selection 160A, but it is only slightly more fruitful.

Presumably, both selections 160A and 365, which occurred as whole-tree variants when discovered in the course of this study, originated as chance limb sports such as 676 and 636, and were accidentally propagated.

SELECTIONS 516 AND 517

In the summer of 1932 it was noted that two parts of a small tree in one of the Corporation orchards at South Haven were bearing very unevenly. Harvest records showed that one side (selection 516) yielded 22 pounds, while the other (selection 517) which had about the same bearing area yielded only 13 pounds. In the spring of 1933 rather severe killing of flower buds by frosts occurred on the nights of April 25 and 26. The percentages killed on the two parts of the tree were 68 and 87, respectively, indicating distinctly greater resistance to frost in selection 516, which had produced the heavier yield

the preceding year. Fruit setting on the two parts of the tree in 1933, as determined from large random samples of uninjured blossoms, amounted to 9 and 1.4 percent, respectively, and the subsequent yields were $1\frac{1}{2}$ and $\frac{3}{4}$ pounds. Practically no winter injury to the flower buds of either part was occasioned by the severe winter of 1933-34, and there was no frost injury in the spring of 1934. Fruit setting on the two parts of the tree amounted to 35 and 8 percent,

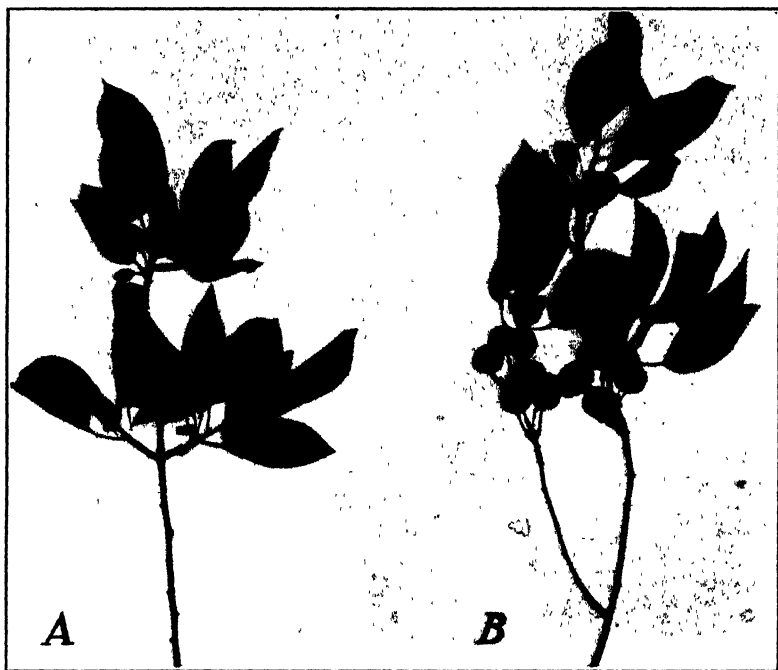


FIGURE 7 Representative fruiting branches of selections 517 (A) and 516 (B) crop of 1934, when fruit setting on the two parts of the tree amounted to 8 and 35 percent, respectively

respectively, and the subsequent yields were 29 and 10 pounds. Figure 7 shows representative fruiting branches from the two halves of the tree.

SELECTIONS 645, 674, 679, AND 686

In the course of this study a considerable number of whole-tree variants, more or less closely resembling limb sport no. 517 or intermediate between it and no. 160A, have been studied. Among these may be mentioned selections 645, 674, 679, and 686.

Selection 645 is a whole-tree variant in the orchard of George Humphrey at Coloma. When first brought to the writer's attention in 1933 it was almost barren, though surrounding trees were heavily loaded. The owner stated that ever since the orchard had reached bearing age, this tree, though blooming profusely each spring, had failed to yield a satisfactory crop. In 1934, fruit setting, as determined from a random sample of 883 flowers, amounted to 2 percent, while records for surrounding normal trees showed a 25-percent set of fruit. The fruit on this tree and its vegetative characters are

unquestionably those of the Montmorency. This is mentioned because of the fact that hand-pollination of a number of the blossoms on this tree with pollen of the Windsor, a sweet cherry variety, yielded a good set of fruit, such as one would expect when using sweet cherry pollen on the blossoms of some variety of the Duke group.

Selection 674 is a whole-tree variant in the orchard of Ralph Rider at Shelby. In 1933 it bore only a very light crop, though surrounding trees of the same age were bearing heavy crops. In the spring of 1934 it blossomed heavily; the blossoms appeared to be normal in every respect, and there was no frost injury in the orchard. Two percent of its blossoms matured fruit while the corresponding percentage on a large composite sample from surrounding trees was 30. The owner was unable to furnish information as to the previous behavior of this tree.

Selections 679 and 686, located in the Gore orchard at Traverse City, could be described in practically the same terms as selection 674, except that the owner had observed for years that these particular trees had been consistently light producers. In 1934, fruit setting, as determined from random samples of 779 and 1,106 flowers, respectively, amounted to 7 and 2 percent.

OTHER INSTANCES

Though many other records have been obtained of both limb and whole-tree variants in the Montmorency cherry characterized by fruit setting that is below normal and resulting in light bearing or in a more or less barren condition, enough have been cited to show at least that they are not rare. Attention should be called, however, to the fact that a number of the variants that are unproductive primarily because of limited flower bud formation, or susceptibility of flower buds to winter cold or to spring frost, are likewise characterized by poor fruit setting. Thus, selection 159, cited earlier in this paper as having flower buds especially susceptible to winter cold, matured fruits from only 6 percent of its flowers in both 1933 and 1934, while the corresponding set on the four surrounding trees was 21 and 22 percent, respectively, and in 1934 the set on one small "reverted" branch on tree 159 was 23 percent. The percentage of blossoms setting fruit on selection 194, a semibarren limb sport characterized by a very small number of flower buds to the spur, were 29 and 27, respectively, in 1933 and 1934, while the corresponding percentages on the normally productive part of the tree were 35 and 43. Selection 703, a limb variant in the orchard of John Stanek, Traverse City, similarly characterized by fruit spurs bearing few flower buds, matured fruit from 2 percent of its blossoms in 1934, while the corresponding percentage on the more normal part of the tree was 34. Selection 680, a limb sport in the Gore orchard, Traverse City, characterized by delayed winter-killing of its flower buds, matured fruit from only 6 percent of its blossoms in 1934, while the corresponding percentage for the remainder of the tree was 11.

Study of the whole mass of records obtained during the past 5 years relating to various aspects of the problem of low productivity in the Montmorency cherry leads to the belief that to the extent that light cropping is due to bud variation, it results more frequently from poor setting of the blossoms than from any one or even all of

the other factors that have been discussed in this paper. Furthermore, the records show that in a considerable number of instances where low productivity is due primarily to one of these other factors, poor setting is an associated or contributing factor. In a relatively large number of instances poor setting alone seems to be responsible for subnormal yields.

DISCUSSION

The worthlessness, commercially, of strains like selections 152, 159, 680, 697, 160A, and 365, that for one reason or another are practically barren, is obvious. Of far greater importance, because less easily recognized, are the relatively unproductive variants, such as selections 629, 700, 262, 512, and 517. Though each season the grower may note that some trees in his orchard are bearing only light crops, he does not mark them and does not realize that many are consistently and regularly light producers. He believes that they are simply alternating between heavy and light cropping, as is so commonly the case with apples. Indeed, many of these light-producing variants, especially those whose low yields are associated with susceptibility of flower buds or flowers to winter cold or spring frost, may bear normally some seasons, though on the whole they are unproductive. The result is that they are not recognized for what they are and average yields are reduced accordingly.

SUMMARY

A number of sports of the Montmorency cherry that result in yields ranging from substantial reductions from the normal to an almost completely barren condition are described.

They are classified as follows: (1) Those failing to form any flower buds visible to the naked eye in positions where flower buds are usually differentiated; (2) those on which leaf buds occur in positions where flower buds are ordinarily formed; (3) those showing marked susceptibility of flower buds to injury from low temperature while in a dormant condition; (4) those showing marked susceptibility to injury from less severe temperatures while in delayed dormant or early post-dormant condition; (5) those showing marked susceptibility of flower buds or opening flowers to spring frost, and (6) those characterized by poor fruit setting.

All these departures from type occur as limb sports or as whole-tree variants, the latter presumably originating as limb variants.

From a commercial standpoint, unproductive variants in this variety due to poor fruit setting are more important than those due to other factors.

There is a rather marked tendency for variants that are unproductive because of one of these six factors to have their yields reduced still further by one or another of the other factors.

Variants producing light yields are more important commercially than those that are almost barren, because they are less easily recognized.

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SOME NORTH AMERICAN PARASITES OF BLOWFLIES¹

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INTRODUCTION

Many are the interrelations between the insects involved in the decay and disintegration of animal carcasses. When an animal dies, if weather conditions are favorable blowflies are attracted immediately, and they deposit their eggs or young larvae, usually in the natural orifices. Ants may begin at once to carry away the newly deposited fly eggs, and later they will attack the larvae in the carcass. After fly larvae are present, beetles appear, and their numbers increase steadily. Some species of these beetles deposit eggs from which develop larvae that feed upon hide, hoofs, and horns; other species produce larvae that prey upon blowfly maggots, and some feed upon both carcass and maggots. The adults of nearly all the species of beetles that frequent carrion feed upon immature stages of flies, some occasionally, others almost exclusively.

There are, in addition, species of Hymenoptera that fly to the carcass and parasitize the blowfly larvae, the young parasite developing within the maggot until finally the fly larva, which in the meantime has transformed into a pupa, is completely consumed. When a parasitized maggot is attacked by a beetle or a beetle larva, both parasite and fly larvae are destroyed. After the carrion is consumed and the blowfly larvae have reached maturity, most of them migrate from the carcass and pupate in the soil in some favorable place a few feet or yards away. Many of these migrating maggots carry within them the developing young of their parasites, and even as they migrate they are further attacked by parasites and predators. Those maggots that remain and pupate unprotected within the carcass are often victims of Hymenoptera which parasitize dipterous pupae.

Other less important predators attack the blowflies in the adult stage. These predators usually prey on other insects as well as on flies.

FACTORS AFFECTING THE INSECTS

Many factors which are at work during these processes affect the blowflies and associated insects. The carcass may be consumed entirely or in part by various mammals or vultures, or it may be buried or burned. Total destruction of course ends the fly problem immediately, while partial destruction proportionately reduces the

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² The investigations of parasites and predators of blowflies were carried out at Uvalde, Tex. The data concerning the distribution of *Alysia* and *Psilodora* in California and Arizona were taken from records made by D. C. Parman. Distribution notes were also made on Arizona and New Mexico species by A. W. Lindquist. The author is indebted to the following specialists for the determination of specimens: Hymenoptera, A. B. Gahan, C. F. W. Muesebeck, L. H. Weld; Diptera, *Sarcophaga*, David G. Hall. The drawings were made by Miss Claudelle Lewis.

number of flies involved. Birds or other animals may also pick out all or a portion of the insects.

Climatic conditions act from the very beginning to influence the insect complex at the carcass. An animal dying in warm weather may be covered with fly blows before death, whereas at lower temperatures oviposition may be retarded for several hours or even for an indefinite period. Conditions that permit one species to oviposit may deter another. Very dry weather, particularly hot, dry winds, will lower the percentage of eggs that hatch. In southern Texas, during the summer months, sun scald is an important factor in causing the death of larvae in exposed carcasses.

The species of Diptera that develop in a carcass vary with the locality, season, size of carcass, and many other factors. Temperatures within the carcass influence differently the oviposition of various species. Often one species of fly, usually the dominant one of the season, is present in large carcasses almost to the exclusion of other flies, while in smaller carcasses a different species may be predominant. Competition occurs between the larvae of blowfly species, particularly in small carcasses; however, the results of some environmental or other factors should not be misconstrued as competition between species. There is a sequence in the infestation of a carcass by the different species of flies, but this is extremely variable. The observations of a distinct sequence frequently mentioned in literature have probably been based, to some extent, on the marked differences in time of emergence of the various species from pupae formed in the same carcass.

Physiological, bacteriological, and chemical conditions develop within the carrion which may hasten or retard the development of one or many species of insects.

Eggs, larvae, and pupae of the flies may be attacked by parasites or predators which may destroy them immediately or ultimately. A predator that destroys a fly larva early in the destruction of a carcass may merely make room for the development of another larva, and if a parasite has oviposited in a maggot that is later destroyed by a predator, the egg is wasted.

Despite the combined action of all these influences, some blowfly larvae mature and migrate. The number, however, is variable, and some factor or combination of factors may decrease the number of maggots maturing or the number of one particular species.

The writer believes that the most important influences on the development of blowflies in large carcasses, in the order of their importance, are: The destruction of the carrion by man or animals, environmental and physiological conditions, the destruction of maggots by parasites and predators, and the competition between the species of Diptera. In the case of small carcasses all these factors are operative, with the insect predators and parasites more effective than in large carcasses.

EXPERIMENTAL METHODS

In making tests with blowfly parasites, particularly with those brought to Uvalde from other localities, it was necessary to keep the insects in close confinement. As none of these imported para-

sites were released, field observations could not be made upon them under Uvalde conditions. In studying developmental periods and host preferences, parasites were placed in pint mason jars on meat infested with blowfly larvae. The jars contained 2 inches of sand in which the larvae could pupate, and were capped with 60-mesh brass strainer cloth. All experiments were conducted in a flyproof insectary to prevent infestation of the meat by other species of flies. Unless otherwise stated, the parasites were confined with the fly larvae, and it should be kept in mind that such experiments, particularly the host tests, were made under laboratory conditions.

For the status tests for blowfly parasites and predators, 4-ounce meat baits in pint mason jars were exposed in the field. Upon pupation of the infested larvae, the jars were capped and brought to the insectary.

A distinction is made in this paper as to size of carcasses. Large carcasses, such as those of cattle, horses, and even goats, are seldom, so far as known, frequented by parasites of blowflies, but around them are thousands of predatory beetles and ants that continually deplete the number of fly larvae. Small carcasses, such as those of rabbits, turtles, and armadillos, are frequented by many parasites and predators. The very small carcasses such as birds and fishes produce the largest percentage of infestation by fly parasites.

SOME INSECTS THAT FEED ON BLOWFLIES

There is given here a list of the insects that have been observed at the Uvalde laboratory to prey on blowflies, though not all of them are of great economic importance in relation to the blowflies.

HYMENOPTERA

Larval parasites	Pupal parasites
Braconidae	Braconidae
<i>Alysia ridibunda</i> Say	<i>Aphaereta muscae</i> Ashm.
<i>Alysia fossulata</i> (Prov.)	Diapriidae
Cynipidae	<i>Trichopria hirticollis</i> Ashm.
<i>Psilodora</i> sp.	Pteromalidae
<i>Xyalosema armata</i> (Say)	<i>Mormoniella vitripennis</i> (Walk.)
<i>Xyalosema</i> sp.	
Chalcididae	
<i>Brachymeria fonscolombei</i> (Du-	
four)	
<i>Eniaca terana</i> Ashm.	

ALYSIA RIDIBUNDA SAY

The parasite described by Say (11, p. 380)² in 1828 as *Alysia ridibunda* (fig. 1) belongs in the family Braconidae and subfamily Alysinae. There is considerable difference in the sizes of the adults, as they range from 5 to 9 mm in length. The body is yellowish red, head black, the wings blackish brown, and the legs blackish. The ovipositor is exerted.

DISTRIBUTION

As indicated by specimens in the United States National Museum *Alysia ridibunda* is rather widely distributed, specimens being la-

² Reference is made by number (italic) to Literature Cited, p. 494.

beled as from New York, Maryland, Virginia, North Carolina, Florida, Michigan, Indiana, Illinois, Kansas, Texas, New Mexico, and Arizona. The insect has not been found at Uvalde, Tex., but a heavily populated area was found in Arizona (Pima, Pinal, Cochise, Santa Cruz, Graham, and Greenlee Counties) and another in New Mexico (Socorro, Luna, and Dona Ana Counties). From 1930 to 1933 this parasite was not collected elsewhere in Arizona or New Mexico. During the same period it was not found in collections from Texas, California, Arkansas, Kansas, Louisiana, Mississippi, Georgia, North Carolina, or Florida, although an extensive search was made.



FIGURE 1.—Adult female of *Alysia ridibunda* Say. $\times 8$.

HABITS

The habits of *Alysia ridibunda* have been studied only under laboratory environment. The insect breeds readily in confinement, if tended properly, and, generally speaking, develops uniformly by broods. The progeny obtained from material collected in 1931 parasitized fly larvae and developed an overwintering generation which, upon emergence in the spring of 1932, produced another generation.

Adults of this species kept in confinement at an average daily mean temperature of 85° F. and furnished with such foods as honey, meat, and fruit lived from 3 to 7 days. It is probable that the life of the adults could be lengthened considerably by keeping them under more natural conditions and at lower temperatures.

The females mate as early as the day of their emergence, and they may be placed either upon meat containing various stages of blow-fly larvae or directly upon mature larvae that have migrated after feeding. The long ovipositor is thrust into any portion of the body of the fly maggot. Ordinarily only one egg is deposited at a single insertion of the ovipositor. Usually the parasite stands directly above the larva, the tip of the ovipositor being forced downward and at the same time forward between the parasite's legs.

DEVELOPMENTAL PERIOD

Eggs of *Alysia ridibunda* hatch in 24 hours at 80° F. Frequently under conditions of confinement as many as 10 parasite eggs may be placed in one host larva. On the second day following hatching there is a marked variation in size of the parasites within each individual host. Nearly always one parasite develops much faster than the others. At the end of 7 days, ordinarily only one parasite remains alive in each host, though in a few instances two have been found. In no case, however, did more than one parasite complete development in a host. The supernumerary parasites die in the first instar. At 80° pupation takes place about the twentieth day, and the adults emerge about 7 days later. The parasitized host larva forms its puparium at the conclusion of the feeding period, after which the parasite completes its development. The latter then pupates within the puparium and lies with its head directed toward the anterior end of the puparium. The adult parasite later emerges by cutting through this end of the puparium.

During 1931 and 1932 about 300 specimens of *Alysia ridibunda* were reared in the laboratory insectary. The earliest emergence from overwintering specimens was on March 19. The last oviposition occurred November 22. All material obtained during November overwintered as larvae or pupae in host pupae of *Calliphora coloradensis* Hough, *Sarcophaga plinthopyga* Wied., or *S. misera* var. *sarracenioides* Ald. In January and early February, at Uvalde, *A. ridibunda* was found hibernating as mature larvae or prepupae. By February 25 they had transformed to the pupal stage.

In the summer broods the shortest developmental period, from egg to adult, was 20 days, the longest 45 days. The average developmental period at an average daily mean of 76° to 80° F. was 27 days, but the average period increased to 45 days when the temperature was 66° to 70°. The overwintering individuals required an average of 151 days for development, with extremes of 121 and 174 days.

HOSTS

Alysia ridibunda was reared in the laboratory from the following species of flies: *Sarcophaga plinthopyga*, *S. misera* var. *sarracenioides*, *Lucilia sericata* Meig., *L. unicolor* Towns., *Calliphora coloradensis* Hough, and *Synthesiomia nudiseta* v. d. W. Attempts to rear the parasite in *Ophyra leucostoma* Wied. and *Cochliomyia macellaria* Fab. were unsuccessful. Twenty-four tests were conducted to determine from which species of flies the parasite could be reared. These tests were conducted as follows: In each test the progeny of a single female fly or the combined progenies of several females of the same species were placed on meat, and while feeding were exposed in a cage containing several mated pairs of *A. ridibunda*. After the fly larvae had matured they were allowed to pupate in sand and from these the emergence of blowflies and parasites was recorded. The results of the tests of hosts of *A. ridibunda* are given in table 1.

TABLE 1.—Emergence of *Aluska ridibunda* from blowfly hosts at Uvalde, Tex., 1931-32

Host	Total tests	Successful tests	Average adult parasites per test	Emergence			
				Total	Blow-flies	Parasites	
	Number	Number	Number	Number	Number	Number	Percent
<i>Sarcophaga</i>	14	7	31	346	131	215	62
<i>Calliphora coloradensis</i>	1	1	37	91	54	37	41
<i>Lucilia sericata</i>	2	2	8	81	66	15	19
<i>Lucilia unicolor</i>	2	1	1	25	24	1	4
<i>Synthesiomia nudiseta</i>	1	1	1	224	223	1	.4
<i>Cochliomyia macellaria</i>	3
<i>Ophyra leucostoma</i>	1
Total or average.....	24	12	22	767	498	269	35

Alysia ridibunda strongly resembles *Brachymeria fonscolombi* in its host preferences, being distinctly partial to *Sarcophaga*, breeding readily in *Calliphora*, to a less extent in *Lucilia*, and very rarely in *Cochliomyia*.

ALYSIA FOSSULATA (PROV.)

Provancher (9, p. 391) based his description of *Alysia fossulata* on a specimen from Los Angeles, Calif. The adult is about 5 mm in length, with the head, thorax, and abdomen black and the legs brown. The wings are transparent but with a slightly brownish cast. The ovipositor does not extend beyond the apex of the abdomen.

DISTRIBUTION

Specimens in the United States National Museum, together with Provancher's record and the writer's recent status work, indicate that the species is present in the low valleys of the coastal area of California. This area extends from Alameda and Santa Clara Counties through Monterey County. In the Salinas Valley of Monterey County the parasite was abundant during 1932. Specimens were collected also in Los Angeles County.

DEVELOPMENTAL PERIOD

At Uvalde, Tex., adults of *Alysia fossulata* reared in the laboratory from material collected in Salinas, Calif., were very active and readily attacked blowfly maggots. Fly larvae were parasitized from November 6 to December 1, 1932, and another generation of 33 parasites was obtained. They did not appear to hibernate during cold weather, for they emerged between January 16 and February 18, 1933. The average developmental period was 80 days with extremes of 71 to 101 days. One lot was reared from *Sarcophaga plinthopyga*, one lot from *Lucilia* sp., and two lots from mixed species of blowflies. Adults of the second-generation parasites lived from 5 to 17 days, unprotected, through a minimum temperature of 14° F. The females attacked blowfly maggots, and oviposition apparently occurred, but no progeny survived.

PSILODORA SP. († *Psilodora rufocincta* Kieffer)

The genus *Psilodora* belongs in the family Cynipidae, subfamily Eucoilinae. In the Uvalde laboratory collection there are specimens of the species studied that range in length from 2.5 to 5 mm. The color of the head and body appears to be black but upon closer examination is found to have a brownish cast; the legs are reddish brown; the antennae are dark brown, those of the female 13-jointed

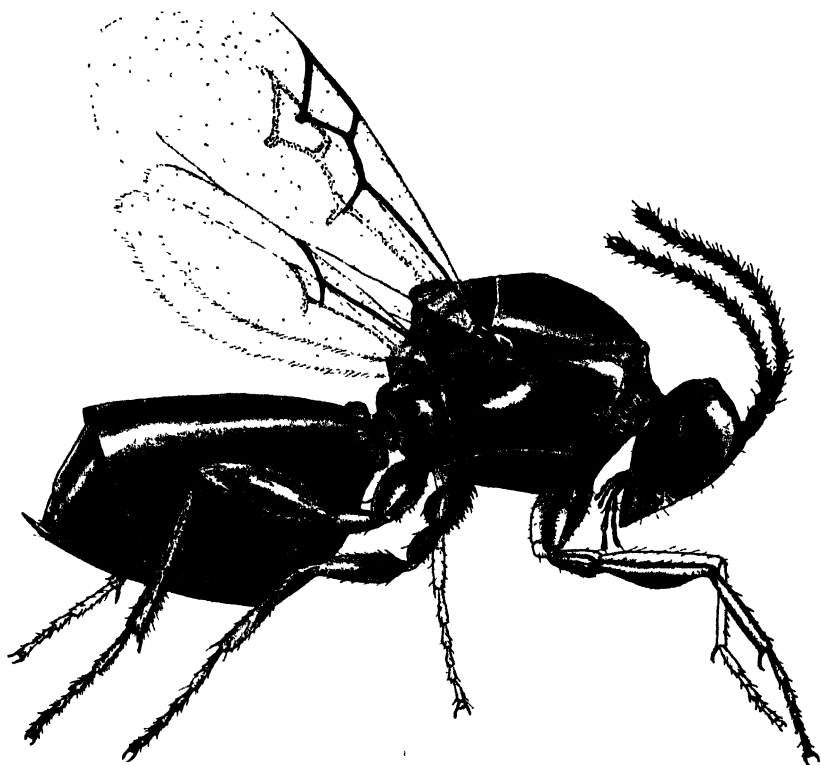


FIGURE 2. Adult female of *Psilodora* sp. $\times 16$.

(fig. 2), subfiliform, pubescent, those of the male 15-jointed, filiform, lightly pubescent; the scutellum has a cuplike elevation above; the abdomen has a hairy girdle at the base.

DISTRIBUTION

This species is confined to the western part of the United States. It was found most abundant throughout Arizona and New Mexico, and to a lesser extent in California (Riverside, Imperial, Kern, Monterey, San Benito, and Santa Clara Counties), Kansas (Pratt and Sedgwick Counties), and Texas (Dallas, Uvalde, and the Panhandle counties). L. H. Weld has observed and collected this species at Oregon Caves, Oreg. This is the most northern record; Dallas, Tex., the most eastern, and Uvalde, Tex., the most southern, in the writer's notes.

HABITS

Psilodora sp. has been recorded frequently as a parasite of maggots breeding in cow dung. The adult of this species has been observed to enter the fresh dung and seek out the maggots, returning to the outside when its work was finished. There is no doubt, however, that under natural conditions this species also infests carrion-breeding maggots, as it has been reared hundreds of times from blowfly larvae in field exposures. The female parasite attacks the larva of the fly, piercing the body wall at any point with her ovipositor and placing one or more eggs in the coelomic cavity. Although all these eggs may hatch, only one parasite completes development. Numerous parasitized pupae have been put in individual glass vials, but not more than one parasite has ever emerged from a single host.

The adults will mate as early as the day of emergence, and about 20 seconds may be consumed in the process of mating. The male will continue to court the female, but is repulsed repeatedly. The female begins oviposition the day of emergence if host larvae are available.

Parthenogenetic reproduction was demonstrated in three tests in which unmated females that emerged in individual glass vials were placed in jars containing blowfly larvae. These larvae, when reared, produced a total of 23 *Psilodora*, sp., all males. The average development period of 35 days was normal for the parasite.

Adults of *Psilodora* sp. in confinement at an average daily mean temperature of 82° F. (extremes 63° to 99°), when given no food or water, lived for an average of 5 days with an extreme of 10 days. When furnished with such food as bananas, figs, or honey, the average longevity was extended to 8 days with an extreme of 18 days. The adult life is probably longer under natural conditions and at lower temperatures.

DEVELOPMENTAL PERIOD

In laboratory tests in 1931 and 1932, 799 *Psilodora* sp. were reared. It is evident from data obtained that this species has a longer developmental period than *Alysia ridibunda* or *Brachymeria fonscolombei*. The overwintering individuals go into hibernation at higher temperatures and remain thus for a longer period; and in the summer broods the developmental period was extremely variable. There is a tendency for some individuals to remain for long periods in the pupal stage. An example of these variabilities is shown by a group of larvae parasitized late in August 1931; from these a number of *Psilodora* emerged, beginning the twenty-eighth day and continuing to the eighty-first day; the remainder of the brood overwintered, some not emerging until 225 days after oviposition. However, in spite of this, *Psilodora* is easily handled and is well adapted for rearing by artificial means.

The earliest emergence of *Psilodora* was recorded for March 2, and the latest oviposition November 22. The records include only broods resulting from ovipositions between March 31 and September 18. The extremes of the developmental periods of the summer broods were 27 and 81 days; and for the overwintering parasites, 112 and 225 days. The average developmental period from egg to adult at

an average daily mean temperature of 71° to 75° F., was 50 days; at from 76° to 80°, 43 days; and from 81° to 85°, 34 days. Overwintering material kept at an average daily mean temperature of from 61° to 65° (minimum, 21°) required an average of 195 days for development.

Psilodora sp. hibernated in 1932 during December, January, and part of February as mature larvae or prepupae in the host puparia. By February 25 the parasite pupae were well developed. Hosts for overwintering *Psilodora* were *Synthesiomyia nudiseta*, *Sarcophaga plinthopyga*, and *Cochliomyia macellaria*.

HOSTS

Thirty lots of blowflies were exposed to *Psilodora* sp. under laboratory conditions and parasites were reared from the following: *Sarcophaga plinthopyga*, *S. misera* var. *sarracenioides*, *S. bullata* Park., *S. pedunculata* Hall, *Lucilia sericata*, *Cochliomyia macellaria*, *Synthesiomyia nudiseta*, and *Ophyra leucostoma*. The results of the tests of hosts for *Psilodora* sp. are given in table 2.

TABLE 2.—Emergence of *Psilodora* sp. from blowfly hosts at Uvalde, Tex., 1931-32

Host	Total tests	Successful tests	Average adult parasites per test	Emergence			
				Total	Blowflies	Parasites	
	Number	Number	Number	Number	Number	Number	Percent
<i>Synthesiomyia nudiseta</i>	2	2	75	210	59	151	72
<i>Sarcophaga</i>	11	5	21	155	50	105	67
<i>Lucilia sericata</i>	7	6	45	521	250	271	52
<i>Cochliomyia macellaria</i>	7	4	6	220	198	22	10
<i>Ophyra leucostoma</i>	3	1	1	75	74	1	1
Total or average	30	18	31	1,181	631	550	47

The greatest number of positive tests was made with *Lucilia sericata*, and 45 parasites per test were obtained, as compared with 21 parasites per test of *Sarcophaga* and 31 for an average of all tests. Apparently *Cochliomyia macellaria* is capable of acting as a host in spite of its short life cycle and the long developmental period of *Psilodora*. Four out of seven tests with this blowfly were successful.

XYALOSEMA ARMATA (SAY)

Xyalosema armata is a member of the family Cynipidae, subfamily Figitinae. The adult insect is 3.5 to 5.5 mm long. The body and head are black with a brownish cast; the antennae are brown except the first segment, which is black; the legs are reddish brown. The antennae in the female are 13-jointed, subfiliform; in the male, 14-jointed, filiform. The scutellum ends in a spine.

DISTRIBUTION

Xyalosema armata is of eastern and middle-western distribution. The writer's collection contains specimens from the following States: Kansas (Pratt, Kingman, Sedgwick, and Sumner Counties), Oklahoma (Grant, Garfield, and Stephens Counties), Texas (Delta

County), Louisiana (Webster Parish), Mississippi (Grenada County), Arkansas (Hempstead, Pulaski, St. Francis, and Crittenden Counties), Illinois (Knox, Henry, Mercer, and Warren Counties), and Florida (Orange County). It is listed among the Hymenoptera of Connecticut (12) and is found no doubt, over all the Eastern States. Its western limits are probably Pratt and the adjoining counties of central Kansas.

HABITS

While this species breeds freely in blowfly larvae and was reared easily in jars containing meat baits in its natural habitat, it could not be reared satisfactorily in this manner at the Uvalde laboratory, although all the common species of blowflies were used. It is probable that the species is averse to breeding in the confinement of jars in the insectary, as trouble was experienced in attempts to rear other species of *Xyalosema*, but it is also possible that the parasite is not adapted to the climate of Uvalde.

Twenty-five host tests with both early instar and mature host larvae were made from May to November, but in two instances only were parasites obtained. *Xyalosema armata* was reared during August from *Sarcophaga bullata*, 27 parasites emerging after an average developmental period of 40 days; and from *S. plinthopyga*, 4 parasites emerging after an average developmental period of 47 days. The average daily mean temperature during these tests was 80° F. This developmental period of 40 to 47 days in *X. armata* is near that for *Psilodora* sp. at the same temperature.

Male and female adults of *Xyalosema armata* if placed together upon emergence will begin courtship immediately. The female will not accept the male a second time, but a male has been observed to mate with six different females within an hour.

The preoviposition period is very brief. A female that emerged one morning was mated at 10 and placed with blowfly larvae from 10:30 until 1 the same day. From these larvae were reared both males and females of *Xyalosema armata*. This demonstrates that fertilized eggs can be produced within 3 hours after the male and female are placed together and in the first half day after their emergence.

XYALOSEMA SP.

Another species of *Xyalosema* is very similar in appearance to *X. armata*, being about the same size and having about the same coloration.

DISTRIBUTION

This species seems rather limited in its distribution. It is abundant along the coast of southern Texas, but has been found in only very small numbers elsewhere. Collections have been made in Texas (Cameron, Uvalde, Val Verde, Lubbock, Hale, and Swisher Counties), Oklahoma (Texas County), Colorado (Weld County), and Mississippi (Grenada County).

HABITS

The parasite was first observed by the author in February 1929 at Brownsville, Tex. Two females were seen upon a 5-pound meat bait that had been exposed to attract blowflies. The parasites were run-

ning about over the meat and down into crevices searching for larvae. They were caught and placed in jars with blowfly larvae; these they parasitized and from them were reared a second generation. This parasite is probably active all winter at Brownsville, as are the blowflies. The parasites were found there again in April 1930, and several were placed upon *Sarcophaga* larvae. A second generation of *Xyalosema* was obtained at an approximate average daily mean temperature of 75° F. with an average developmental period of 55 days. During the same period a small number of males were reared from larvae of *S. plinthopyga* parasitized by unmated females, thus demonstrating parthenogenetic reproduction.

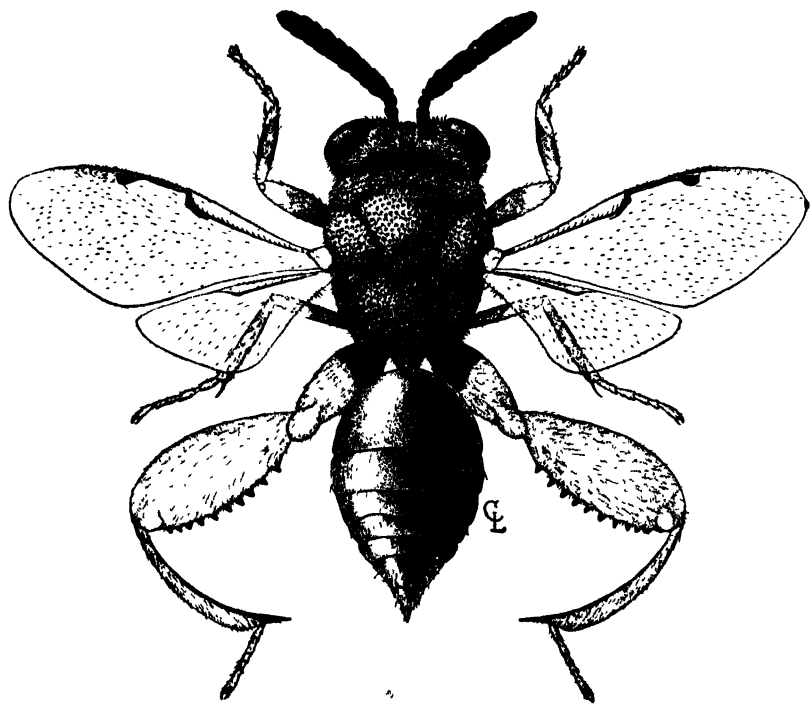


FIGURE 3.—Adult female of *Brachymeria fonscolombei* (Dufour). $\times 12$.

From blowfly larvae taken in a meat bait exposed under field conditions at Uvalde from August 9 to 19, 1932, five *Xyalosema* sp. were reared with an average developmental period of 31 days. These specimens were placed in turn upon six unmixed lots of *Lucilia*, *Cochliomyia*, and *Sarcophaga* larvae, but another generation of parasites was not obtained.

BRACHYMERIA FONSCOLOMBEI (DUFOUR)

Brachymeria fonscolombei belongs to the superfamily Chalcidoidea, family Chalcididae. The adult insect (fig. 3) ranges from 3 to 6 mm in length; the head and body are black, the antennae black, and the legs red with yellowish-white markings; the posterior femur is enlarged, oval, and armed on the lower side with 10 teeth.

DISTRIBUTION

The distribution of *Brachymeria fonscolombei* in the United States seems to be limited only in a northerly direction. Specimens in the writer's collection are from California, Arizona, New Mexico, Texas, Oklahoma, Kansas, Arkansas, Louisiana, Mississippi, Illinois, Maryland, and Florida. Galesburg, Ill., is the most northern record in the writer's notes.

HABITS

The biology of *Brachymeria fonscolombei* has been discussed fully by the author elsewhere (10). This parasite attacks either mature or early instar larvae of blowflies, and not more than one adult emerges from each blowfly pupa. The preoviposition period is brief; females will mate the day of their emergence and produce fertile eggs. Without food or water, at 70° to 79° F., adults live about 4 days. Honey or fruits, when given as food, increase the average longevity to as much as 11 days.

DEVELOPMENTAL PERIOD

The developmental period from egg to adult averages 21 days at an average daily mean temperature of 86° to 90° F. At lower temperatures this period increases until at 65° the average developmental period is 35 days. As the temperature drops still lower a portion of the brood will overwinter as last instar larvae in the host pupae. The developmental period of *Brachymeria fonscolombei* is considerably shorter than that of *Psilodora* or *Xyalosema*.

HOSTS

Brachymeria fonscolombei has been reared from the following species of field-collected and laboratory-reared blowflies: *Calliphora coloradensis*, *Lucilia sericata*, *L. unicolor*, *Phormia regina* Meig., *Cochliomyia macellaria*, *Sarcophaga plinthopyga*, *S. impar* Ald., *S. haemorrhoidalis* Fall., *S. carnaria* L., and *Synthesiomyia nudiseta*.

It is most active as a parasite of *Sarcophaga*. However, it readily parasitizes *Synthesiomyia* and *Phormia* and is frequently reared from *Lucilia* and *Calliphora*. The parasite was rarely reared from *Cochliomyia macellaria*, and it was demonstrated that ordinarily when *Brachymeria fonscolombei* parasitizes this fly both host and parasite larva die.

Brachymeria fonscolombei is found breeding in host larvae in carcasses of birds, rabbits, turtles, and other small animals. At Uvalde about one-third of the blowfly larvae in small carcasses are parasitized.

ENIACA TEXANA ASHM.

Eniaca texana, belonging to the family Chalcididae, subfamily Chalcidinae, has been reared in two instances from blowfly larvae.

On July 10, 1932, a 4-ounce meat bait was exposed in a small bush in a rather open location near Uvalde. The bait was brought in on July 20, and from the blowfly larvae infesting it were reared seven specimens of *Eniaca texana* after a developmental period of about

22 days. The host pupae, *Sarcophaga plinthopyga*, were confined separately in glass vials, and a single parasite was seen to emerge from each infested pupa. These parasites were placed with larvae of *Lucilia*, *Sarcophaga*, and other blowflies, but another generation was not obtained.

Blowfly larvae of various species were exposed at Encinal, Tex., April 5, 1933, and from these were reared five specimens of *Eniaca texana*. The developmental period of this lot was approximately 30 days.

APHAERETA MUSCAE ASHM.

Little is known of *Aphaereta muscae*, which belongs to the family Braconidae, subfamily Alysiinae, except that it emerges occasionally from baits containing blowfly pupae.

Viereck (12, p. 214) states that the insect "occurs" in Connecticut in July and August and that it has been reared from the onion maggot. Leonard (7, p. 919) lists it as common in New York and throughout the Eastern States. The author has reared it from blowfly pupae exposed by H. H. Stage at Salmon River Junction, Oreg., and has collected it over fresh cow dung at Victoria, Tamaulipas, Mexico. At Uvalde, Tex., it has been reared from a mixed lot of blowfly pupae, but in such large numbers (100 in a 4-ounce bait) as to suggest that it was breeding in *Sarcophaga* or *Lucilia*.

TRICHOPRIA HIRTICOLLIS ASHM.

Trichopria hirticollis is a parasite of blowfly pupae and belongs to the family Diapriidae, subfamily Diapriinae. It is encountered at Uvalde, Tex., each year from July to October. In one instance it emerged in April. From adult parasites collected in the field another generation can be reared easily in confinement.

No accurate data have been collected on the life history of this parasite, but it has been reared several times from *Sarcophaga plinthopyga* after a developmental period of 25 to 30 days. The average developmental period is probably shorter than this. As *Trichopria hirticollis* emerges at the same time as *Mormoniella vitripennis* from material exposed in the field, it is probable that their developmental periods are of about the same length.

From one lot of parasitized blowfly pupae of miscellaneous species, an average of 23 *Trichopria hirticollis* per host puparium was obtained. The maximum was 44 parasites from one puparium. In another test 32 parasites were secured from one puparium of *Sarcophaga plinthopyga*.

MORMONIELLA VITRIPENNIS (WALK.)

Mormoniella vitripennis (*Nasonia brevicornis* Ashm., *Pteromalus abnormis* Boh.) belongs to the family Pteromalidae. Girault and Sanders (4) in redescribing *M. vitripennis* give the following data concerning its size and color. Female: length, very variable, 1.0 to 2.30 mm, average 1.75 mm; color, metallic dark brassy green. Male: length, very variable, 0.60 to 2.0 mm, average 1.32 mm; color, lighter than female, more brassy, metallic, and green.

This small parasite of blowfly pupae is practically world-wide in its distribution. Gahan (3) states that in the United States it

occurs from New Jersey and North Carolina to California. It is probably the most important of the parasites attacking blowfly pupae, but even so it is of no great economic importance. The large numbers of this insect seen about carrion and the readiness with which it will attack pupae are misleading as to its value. Of 79,604 blowfly larvae reared from 1929 to 1932 at Uvalde, only 0.7 percent were parasitized by *Mormoniella vitripennis*. This is probably about its normal parasitization of blowflies. During the year of its maximum abundance since these studies have been under way, those reared amounted to only 1 percent of the total emergence of flies and parasites. As with other parasites of pupae it can act only on those flies which pupate in a fairly exposed place. At Uvalde, the chief value of this parasite is in its parasitization of the few pupae of fly larvae that fail to migrate and pupate within the carrion. One hundred percent of such pupae may be parasitized.

Various authors give the length of the life cycle as 11 to 27 days. At Uvalde, in the summer months, the parasite develops from egg to adult in an average of 14 days, the developmental period lengthening with cooler weather. *Mormoniella vitripennis* overwinters in the host puparium as mature larvae or as pupae, and overwintering specimens have required as long as 140 days for development. The species is inclined to continue breeding, however, whenever temperatures are favorable, and it is likely to begin emergence on any warm day during the winter. It has hibernated successfully in pupae of *Cochliomyia macellaria* as well as in other blowflies.

From observations at Uvalde, and from records of other workers, it appears that *Mormoniella vitripennis* will infest pupae of all the species of blowflies and of many other Diptera. At Uvalde it has been reared from *Cochliomyia macellaria*, *Phormia regina*, and numerous species of *Lucilia*, *Sarcophaga*, and *Calliphora*, both from field-collected and laboratory-reared hosts. The author has reared it from the cheese skipper, *Piophilæ casei* L.; Bishopp et al. (1) reared it, although not under field conditions, from *Hypoderma lineatum* De Vill.; Fujita (2) found it infesting *Tricholyga bombycis* Bech., a tachinid parasite of the silkworm; and Johnson (6), in discussing injury to nestling birds by *Protocalliphora*, refers to several instances of parasitization of *Protocalliphora* by *M. vitripennis*.

It is important to know the number of *Mormoniella vitripennis* which normally develop within each host puparium in order that when a given number of pupal parasites have emerged, the number of host pupae destroyed may be estimated. The number of parasites per fly puparium is dependent primarily on the host species. Hardy (5) states that 40 parasites may be reared from a single puparium, but that an average of 7 emerge from a single puparium of *Lucilia*. Fujita (2) obtained 37 parasites per puparium from the tachinid *Tricholyga bombycis*. At Uvalde, an average of 36 *Mormoniella vitripennis* was obtained from each puparium of *Sarcophaga plinthopyga*. An average of 46 (maximum 85) *Mormoniella vitripennis* were reared from a lot of 33 pupae of *Sarcophaga* spp., and an average of 36 parasites per puparium from *Phormia regina*.

ADDITIONAL NOTES ON PARASITES

There are a few other parasites that have been infrequently collected or of which the exact status is not known. In July 1930 numerous specimens of a species of *Xyalosema* were reared from *Cochliomyia macellaria* at San Luis Potosi, Mexico, by R. C. Mundell. In April 1931, however, extensive collections by the author at San Luis Potosi revealed none of these parasites, probably because it was too early in the year.

A specimen of *Eucoila* sp. was found on a meat bait containing blowfly larvae at Washington, D. C., in July 1932.

Pachycrepoides dubius Ashm. is world-wide in distribution and is recorded from many parts of North America as a parasite on Sarcophagidae, Muscidae, dung flies, and other Diptera. Numerous specimens were reared by D. C. Parman at Ulvade in October 1928 from pupae in a meat bait.

In May 1930, 90 specimens of *Muscidifurax raptor* Gir. were reared from a lot of over 1,000 pupae of *Musca domestica* L. collected by F. C. Bishopp in April at El Paso, Tex. In the writer's collection are specimens of *M. raptor* reared in September 1914 by W. E. Dove at Dallas, Tex., from pupae of Diptera breeding in garbage.

Spalangia muscidarum Richardson is commonly reared from *Stomoxys calcitrans* L., as discussed by Pinkus (8). This species and others of the same genus appear frequently in breeding work with blowflies. The author has reared it in two instances while working with *Lucilia sericata*. In each instance, however, it emerged in very small numbers and might have been breeding in very small Diptera (*Aphiochaeta iroquoiana* Mall.) which were present in the meat.

SUMMARY

Blowflies breeding in carcasses are affected by the following factors: (1) Destruction of the carrion by man or animals, (2) environmental and physiological conditions, (3) destruction of maggots by parasites and predators, and (4) competition between the species of Diptera. Hymenopterous parasites are most active in small carcasses such as rabbits, turtles, and birds.

Species of the genera *Alysia*, *Psilodora*, *Xyalosema* and *Brachymeria* attack blowfly larvae. These species deposit their eggs within bodies of fly maggots and produce but a single parasite in each host. *Brachymeria* and *Alysia* breed most readily in *Sarcophaga* but are reared also from *Calliphora*, *Lucilia*, and other blowflies. *Psilodora* and *Xyalosema* have a wider range in host selection and breed to some extent in *Cochliomyia*. *Alysia* and *Psilodora* were found in abundance in the Southwestern States, although occasional eastern and northern records are known. *Brachymeria* was found throughout the southern and central parts of the United States. *Xyalosema* is mainly of eastern distribution, although one species is found in the Texas coastal region. All of these parasites are of value because of the extent of their parasitism of blowfly larvae in carcasses. Especially important are those known to attack *Cochliomyia*.

Of the parasites which affect blowfly pupae, *Mormoniella* is the most important. *Aphaereta* and *Trichopria* are commonly encountered. These parasites produce a number of offspring in a single

host pupa and may easily be reared in large numbers under laboratory conditions. Their effectiveness in blowfly control is not great, however, because most fly larvae pupate in the soil where they are well protected from parasites.

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BRACHYMERIA INTERMEDIA (NEES), A PRIMARY PARASITE, AND B. COMPSILURAE (CWFD.), A SECONDARY PARASITE, OF THE GYPSY MOTH^{1 2}

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INTRODUCTION

Brachymeria intermedia (Nees) is a European species parasitic on the gypsy moth (*Porthetria dispar* L.), as well as many other species of Lepidoptera. It was reared as early as 1905 from gypsy moth pupae sent to the Bureau of Entomology laboratory at Melrose Highlands, Mass. Since that time several unsuccessful attempts have been made to establish it in New England.

Brachymeria compsilurae (Cwfd.) is a parasite of tachinid flies which is apparently native to North America. It probably was of slight economic importance until after the establishment in New England of two European tachinids, *Compsilura concinnata* Meig. and *Sturmia scutellata* R. D., parasitic on the gypsy moth. Crawford (6)³ first described it in 1911, and five of his paratypes were reared from *C. concinnata*. It is now recognized as an important enemy of both these beneficial insects.

Some observations on *Brachymeria compsilurae* were begun in 1924, but this work was interrupted in 1925 and also from 1928 to 1931, inclusive, while the writer was stationed in central Europe. The work in Europe consisted in collecting and shipping beneficial insects to the United States, and provided an opportunity for studying *B. intermedia*. It soon became apparent that, although there is a close morphological relationship between the adults of *B. intermedia* and *B. compsilurae*, there is a radical difference in the bionomics of the two species; furthermore, their immature stages exhibit marked differences. Because both of them may be important factors in the control of *Porthetria dispar*, it was decided to present the results of investigations on both species in a single paper.

LITERATURE ON BRACHYMERIA

Until recently the genus *Brachymeria* has been known as *Chalcis*, and nearly all references in the literature are under the genus *Chalcis*. It is a large genus, including more than 150 described species. Most of these species are found in tropical or subtropical climates, although many are common in the Temperate Zone, and some have a very wide distribution. *B. fonscolombei* (Dufour) is well distributed over Europe

¹ Received for publication Jan. 8, 1935; issued May 1935.

² This study was conducted at the Melrose Highlands, Mass., laboratory of the Bureau of Entomology during 1924, 1932, and 1933, and at its European sublaboratory, Budapest, Hungary, during 1929 and 1930. The writer is indebted to C. W. Collins, in charge of the Melrose Highlands laboratory, for making these investigations possible; to C. F. W. Muesebeck, D. L. Parker, and T. H. Jones for helpful advice and the use of notes which each of them made on these species; and to W. F. Sellers for help in collecting material in Europe.

³ Reference is made by number (italic) to Literature Cited, p. 522.

and North America and has been found in Asia. *B. ovata* (Say) is common throughout the United States and the West Indies, and has recently been taken in Argentina. Many of the species are primary parasites of Lepidoptera. Pests of some of our most important crops, including cotton, sorghum, bananas, coconuts, and sugarcane, are heavily parasitized by members of this genus. A few species are definitely hyperparasitic, attacking tachinid parasites of Lepidoptera and sarcophagid parasites of Orthoptera; and one species, *B. secundaria* (Ruschka), has been noted as a secondary parasite of Hymenoptera. Several species attack sarcophagid flies which feed on carrion; and two species, *B. ritripennis* (Först.) and *B. euthyrrhini* (Dodd), are parasites of Coleoptera.

In spite of the size and importance of the genus, only one species, *Brachymeria fonscolombei*, has been studied in detail. As early as 1840 Dufour (7) described this species (under *Chalcis*) and gave an account of its life history. In 1923 Parker (19) gave further notes on its biology, and in 1924 he (20) fully described and illustrated the immature stages. In 1933 Roberts (24) published a paper on the biology of *B. fonscolombei* as a parasite of blowfly larvae.

Short accounts of several other species of *Brachymeria* have been published, however. *B. intermedia* was discussed briefly, under the name *Chalcis flavipes*, by Howard and Fiske (11) in 1911. A short resume was given of the parasite's life history, with figures of the full-grown larva, pupa, adult, and exit holes in *P. dispar* pupae. Burgess and Crossman (1, p. 116) also outlined its life history in 1929. They summarized rearings of *B. intermedia* from *P. dispar* pupae in Europe, and recorded the number of adults liberated in the United States; they also discussed the possibility of the establishment of the parasite in New England, and reviewed the host relations of the species. In 1924 Silvestri (28, pp. 81-82) published notes on this species as a parasite of *Tortrix viridana* L. In 1931 Martelli (13, pp. 205-207) gave a brief account of the biology of *B. intermedia* var. *scirropoda* (Först.), a parasite of *Aporia crataegi* L. In 1889 Stefani (29) published a short paper on (*Chalcis*) *Brachymeria dalmani* (Thoms.) as a parasite of sarcophagids. In 1929 Olsoufieff (17, pp. 95-99) discussed the same insect as a parasite of four species of sarcophagid flies parasitic on the Asiatic locust (*Locusta migratoria* L.) in Russia. He described the adult, the fifth-stage larva, and the pupa, and gave notes on its life history. Rukavishnikov (25) reported on the same species as a parasite of the same sarcophagids in 1930.

In 1897 Howard (10) published a short account of (*Chalcis*) *Brachymeria ovata* (Say) as a parasite of (*Orgyia*) *Hemerocampa leucostigma* S. and A., including figures of the adult, pupa, pupal exuvia, and exit hole in a parasitized pupa. Howard and Fiske (11, p. 242) in 1911, and Burgess and Crossman (1, p. 118) in 1929, gave brief resumes of the life history of (*Chalcis*) *Brachymeria obscurator* (Walk.). In 1923 Paillot (18) wrote a short paper on (*Chalcis*) *Brachymeria femorata* (Panz.), a parasite of *Pieris brassicae* L., and in 1926 Faure (8, pp. 72-75) gave notes on the biology of the same species, figuring the adult, the full-grown larva, and the head and mandible of the full-grown larva.

Nothing has been published on the biology of *B. compsilurae*, but in 1934 Proper (22) noted it as a parasite of several tachinid flies, giving percentages of puparia infested.

BRACHYMERIA INTERMEDIA (NEES)

DISTRIBUTION AND HOST RELATIONSHIPS

Brachymeria intermedia is well distributed throughout southern Europe and northern Africa. Rearings of gypsy moth material in connection with the work of the Bureau of Entomology show recoveries from Portugal, Spain, France, Italy, Hungary, Czechoslovakia, Yugoslavia, and Algeria. De Lépiney (12) records it from Morocco, Chorbadzhiiev (2) from Bulgaria, Masi (14, pp. 78-82) from Rumania, Rusehka (26) from Turkey and East Bokhara, and Sacharov (27) from southern Russia (Astrakhan).

B. intermedia is perhaps best known as a parasite of *Porthetria dispar*. It is, however, very polyphagous, attacking many species of lepidopterous pupae and at least one species of hymenopterous cocoon. Under laboratory conditions it has also been reared from several species of tachinid puparia. In the literature it has only been recorded as a parasite of lepidopterous pupae. A list of the recorded host species follows:

Aporia crataegi L. (26)
Hypogymna morio L. (26)
Malacosoma neuustria L. (9, 23)
(Oenophtheria) Sparganothis pilleriana
 Schiff. (26, 30)
Papilio podalirius L. (26)
Pieris daphidice L. (27)⁴
Pieris rapae L. (27)
Polia oleracea L. (15)

(*Tmetocera*) *Eucosma ocellana* F. (13, 28)
Tortrix pronubana Hbn. (3)
Tortrix viridana L. (3, 9, 28)
Zygaena angelicae O. (26)
Zygaena ephialtes L. (26)
Zygaena filipendulae L. (13, 14, 26, 28)
Zygaena transylvanica Burgeff (13, 14, 28)

During the present study it has been reared from pupae of *Vanessa io* L., and also from cocoons of the hymenopterous parasite *Rogas unicolor* Nees. *R. unicolor* is a parasite of *Stilpnotia salicis* L. in central Europe. Its cocoon is formed within the larval skin of the host, which becomes a dry, taut case. Adults issue in from 10 to 14 days after formation of the cocoon, and during this period it is exposed to the attack of hyperparasites. In 1933, 2,254 *Rogas* cocoons were collected in Budapest, Hungary, and 2 of them produced *B. intermedia*. In 1934, 4,869 *Rogas* cocoons were collected, and *B. intermedia* issued from 12 of them.

A study of the host relationships of *B. intermedia* was made under laboratory conditions at Budapest, Hungary, by exposing to attack numerous lepidopterous pupae, tachinid and sarcophagid puparia, and hymenopterous cocoons. Practically any naked lepidopterous host pupa was attacked, although hairy pupae, such as *Stilpnotia salicis*, were refused. All hymenopterous cocoons were refused, and sarcophagid puparia were apparently too hard for the females to pierce. Tachinid puparia, on the other hand, were readily attacked, especially when fresh. A list of host species in which *B. intermedia* was reared in the laboratory follows:

Lepidoptera:

Abrostola triplasia L.
Acronycta tridens Schiff.
Cadonia punctoria L.
Cynthia atalanta L.
Mamestra brassicae L.
Nygmia phaeorrhoea Don.
Pieris brassicae L.
Polygonia c-album L.

Lepidoptera—Continued

Vanessa xanthomelas Esp.
Vanessa urticae L.

Diptera (tachnid puparia):

Compsilura concinnata Meig.
Sturmia bella Meig.
Sturmia inconspicua Meig.
Tachina larvarum L.
Tricholyga sorbillans Wied.

⁴ Faure (8, p. 75) believes Sacharov's species may be *B. femorata* (L.).

Brachymeria intermedia has also completed development in the following American species attacked at the Melrose Highlands laboratory:

Autographa brassicae Riley
Basilarchia archippus Cram.
Cynthia atalanta L.
Euphydryas phaeton Dru.
Hamadryas milberti Godt.

Hemerocampa leucostigma S. and A.
Heterocampa guttivitta Walk.
Lemonias harrisi Scudd.
Papilio glaucus L.

Although *B. intermedia* has been reared from field-collected hymenopterous cocoons, and it readily parasitizes tachinid puparia in the laboratory, the large number of lepidopterous hosts recorded in the literature makes it seem certain that under field conditions it is almost entirely a parasite of that group. Apparently it is rarely a parasite of *Rogas unicolor*. It may attack other species of Hymenoptera, but no such records have been made. Difficulties encountered in collecting tachinid puparia in the field have made it impossible to judge whether *B. intermedia* ever attacks these hosts under natural conditions, but even though it readily attacked and completed development in tachinid puparia that were exposed to it in the laboratory, it is not believed to be normally a parasite of tachinids. Several attempts have been made to induce it to attack tachinid maggots in lepidopterous pupae and larvae, but without success; and since most tachinids form their puparia in the soil, they would be inaccessible to *B. intermedia*.

ECONOMIC IMPORTANCE

Most of the rearing work to obtain parasites of *Porthetria dispar* that has been conducted in Europe by the Bureau of Entomology has been in central European countries, and there *B. intermedia* has been of slight importance as a control factor. Twice, however, it has been observed to be extremely important in more southern rearing points. In 1911 W. F. Fiske⁶ made large shipments of parasites from a heavy infestation of *P. dispar* at Caltagirone, Sicily. He considered *B. intermedia* one of the best parasites at that point, noting that 4 or 5 adults could be observed flying around each tree. He gave no figures on the percentage of pupae parasitized, but he had 44,000 "parasitized" pupae and 12,000 adults collected, and these figures indicate that the species must have been plentiful. In 1927 R. T. Webber⁶ conducted rearing work in a forest at Oran, Algeria. He considered it "undoubtedly the most important parasite" in that forest. A collection of 100 host pupae yielded no less than 26 *B. intermedia*. Pupae that were shipped to the United States from Oran unfortunately suffered severe mortality; so it was impossible to estimate the degree of parasitization of a large number of pupae. In 1930 De Lépiney (12, p. 87) gave a detailed account of a study of *P. dispar* in the forest of Mamora in Morocco. He makes the following remarks regarding *B. intermedia*.

Chalcis intermedia was of primary importance in certain regions of the forest in 1924 and 1925. In 1925 nearly all the pupae along the Salé-Tiflet road in the region designated as "promontory A", which was totally defoliated, were parasitized, so that *P. dispar* was rarely seen in 1926; total defoliation was not observed again in that region until 1928. But since 1925 *Chalcis intermedia* has practically disappeared, not only on promontory A, but throughout the whole forest; only a few specimens having been found.

⁶ Unpublished notes at the Melrose Highlands laboratory.

Rearing work has also been conducted in Spain and Portugal. The climate of these countries is probably suited to *B. intermedia*, but the parasite was of minor importance during the 2 years in which collections were made. In 1924, 7,683 *P. dispar* pupae were collected in Spain, and 315 *B. intermedia* adults were recovered. In 1925, 957 *B. intermedia* adults were reared from 25,000 host pupae collected in Spain, and 1,433 from 51,900 pupae collected in Portugal.

From these facts we may conclude that *B. intermedia* occurs generally as a parasite of *P. dispar* in the Mediterranean countries, and that at times it becomes of primary importance as a control factor. Apparently, however, it does not thrive in the colder climate of central Europe, where the mortality among overwintering adults probably is very high. This seems to be equally true in New England for, although more than 20,000 adults have been liberated, the parasite has failed to establish itself in spite of the fact that it has been reared in the laboratory on various American host species.

Sacharov (27) found *B. intermedia*⁶ to be an important parasite of the mustard feeder, *Pieris daphidice* L. In 1911 it was responsible for the destruction of about 50 percent of the pupae of this species where studies were conducted in the Government of Astrakhan in southern Russia.

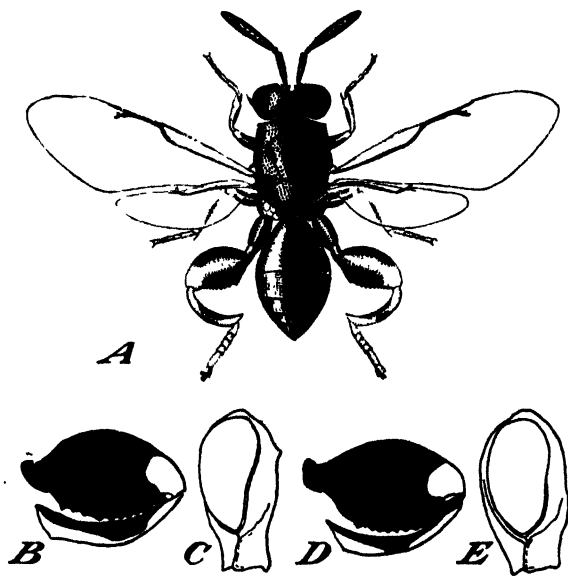


FIGURE 1—*Brachymeria intermedia*: A, Adult (from Howard), (X 6); B, adult female, hind femur and tibia, showing markings (from Crawford) (X 11); C, head, showing carina at front of malar space (from Crawford) (X 15); D, adult female, hind femur and tibia, showing markings (X 11); E, head showing carina at front of malar space (X 15).

THE ADULT

(*Chalcis*) *Brachymeria intermedia* (fig. 1, A) was first described by Nees in 1834 (16, pp. 29–30). Masi (14, pp. 78–82) redescribed it in 1916, and Ruschka (26) wrote a redescription in 1922. Crawford (5) keyed the species (under the name *Chalcis flavipes* Panz.) in 1910, and his figures of the “hind femur and tibia, showing markings” and “head, showing carina at front of malar space”, are reproduced in figure 1, B and C.

THE EGG

The newly laid egg (fig. 2, B) is 0.90 mm long and 0.20 mm wide. It is slightly curved with rounded ends, being somewhat wider at

⁶ See footnote 4, p. 497.

the cephalic end. The chorion is smooth and hyaline except for a slightly roughened area at the micropyle. The embryo is semi-opaque and grayish in color.

THE FIRST-INSTAR LARVA

The first-instar larva is strikingly different from that of *Brachymeria compsiturae* Cwfd. It closely resembles ectoparasitic forms in

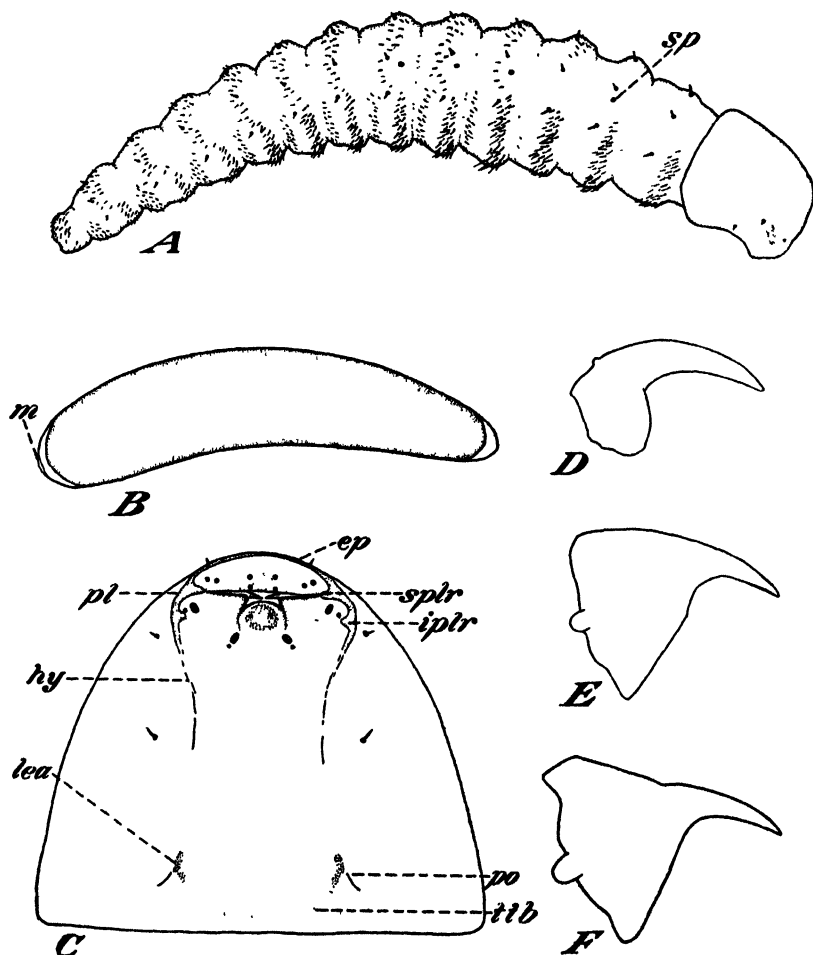


FIGURE 2.—*Brachymeria intermedia*: A, First-instar larva (× 92); sp, spiracle. B, Egg (× 67); m, micropyle. C, Head of first-instar larva, ventral aspect (× 275); ep, epistoma; splr, superior pleurostomal ramus; plr, pleurostoma; iplr, inferior pleurostomal ramus; hy, hypostoma; lea, lateral epicranial angles; po, postocci-put; ttb, transverse tentorial bar. D, First-instar mandible (× 500). E, Second-instar mandible (× 428). F, Third-instar mandible (× 300).

having 13 well-differentiated segments, and is tapered posteriorly. It belongs to Parker's group 6 (20).

The newly hatched larva measures about 1.08 mm long and 0.23 mm wide, with body form as shown in figure 2, A. The head is well defined, and there are 13 body segments about equal in length. Each

segment bears a number of cuticular spines (fig. 3, *C*), and all except the last have 3 pairs of sensory setae (fig. 3, *B*) placed as follows: 1 just above the line of spiracles, 1 near the dorsal margin of the segment, and 1 just below the median line. Possibly the last abdominal segment has sensory setae, too, but none could be distinguished. The sensory setae are much like the cuticular spines, especially on the posterior segments. Below the median line the spines on the anterior portions of the segments are directed backward

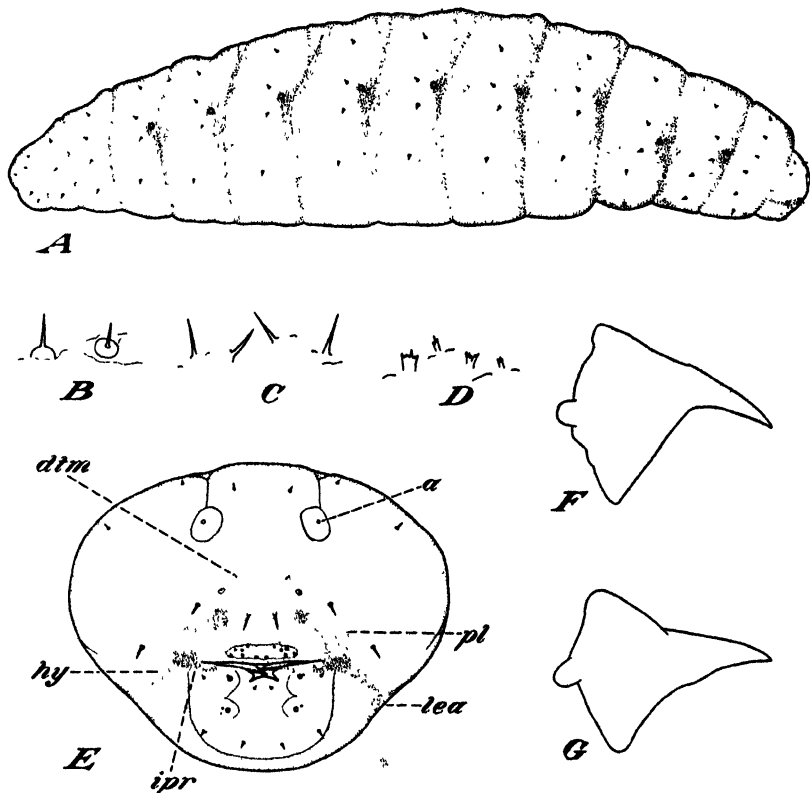


FIGURE 3—*Brachymeria intermedia*: A, Full-grown larva (X10). B, Sensory setae (X400). C, Cuticular spines (X400). D, Compound cuticular spines of last abdominal segment, fourth instar (X400). E, Head of full-grown larva; *a*, antenna; *dtm*, dorsal tentorial macula; *pl*, pleurostoma; *hy*, hypostoma; *ipr*, inferior pleurostomal ramus; *lea*, lateral epicranial angles (X50). F, Fourth-instar mandible (X200). G, Fifth-instar mandible (X120).

and those on the posterior portions are directed forward. Above the median line all the spines are directed backward. The last abdominal segment differs from the others in having no well-defined groups of spines, although spines are scattered over the entire surface, all of which are directed backward.

The head is thimble shaped and lightly sclerotized (fig. 2, *C*). It bears a pair of short truncate antennae. The mouth opening is on the ventral side at the apex. The mandibles (fig. 2, *D*) are 0.05 mm long. They are sickle shaped, well sclerotized, and yellow at the tips. There is a skeletal crossbar (not shown in the illustration) connecting the inferior pleurostomal rami and extending across the

mouth opening just below the mandibles. Phillips (21) calls this crossbar the "hypopharyngeal bracon." The hypostoma is weak except at the epicranial angles and just below the pleurostoma, the postociput is poorly defined, and the transverse tentorial bar is weakly sclerotized.

The tracheal system is composed of a pair of lateral longitudinal trunks united anteriorly in the first thoracic and posteriorly in the ninth abdominal segment. There are 4 pairs of open spiracles, a pair on the second thoracic and on each of the first 3 abdominal segments. They measure 0.006 mm in diameter. Rudimentary spiracular branches are present in the third thoracic and the fourth to seventh abdominal segments, although there are no open spiracles on these segments. The spiracular branches give rise to several dorsal branches in each segment, as well as several that are directed ventrally. The main ventral branches arise from the longitudinal trunk near the base of the spiracular branches. These subdivide into many smaller branches. The head is supplied from branches arising in the first thoracic segment, and the last abdominal segment receives branches from the longitudinal trunks in the ninth abdominal segment.

The digestive system is well developed at the time of hatching. The fore-intestine extends to the middle portion of the first thoracic segment, where it empties into the mid-intestine, or stomach. The mid-intestine occupies most of the body cavity, extending back to the anterior portion of the eighth abdominal segment, where it joins, but does not open into, the hind-intestine. The hind-intestine has a narrow anterior portion, to which the malpighian tubes are attached, and a wider, urn-shaped rectum. The salivary glands are conspicuous tubes lying on each side of the body. They unite in the posterior portion of the head into a common duct which opens on the floor of the mouth. The tubes are nearly straight in the first two thoracic segments, but they then become strongly serpentine, making a large curve in each segment as far back as the seventh abdominal, where they straighten out and extend along the venter of the eighth abdominal segment, turning upward and terminating in the ninth. There are two pairs of malpighian tubes connected to the base of the hind-intestine. A short, but strongly sinuous dorsal pair extends backward into the last segment. The other pair are long tubes extending anteriorly just above the salivary-gland tubes. They are attached to the hind-intestine just below the dorsal pair; from this point they curve upward and then extend in a nearly straight line to the third thoracic segment.

The brain and nerve cord are distinct. The latter is relatively wide, with only slight swellings at the ganglia. It extends to the seventh abdominal segment, where the last 2 pairs of ganglia are fused, giving off 2 pairs of nerve fibers.

The dorsal blood vessel extends from the head to the ninth abdominal segment. It is a muscular, tubelike organ lying along the dorsal wall. It is difficult to distinguish in the first instar, but may be seen readily in older larvae.

THE SECOND-INSTAR LARVA

Second-instar larvae are from 2.00 mm long and 0.53 mm wide to 2.86 mm long and 0.88 mm wide. They are similar to the first-instar

larvae, but are readily distinguished by the shape of the mandibles and the size of the spiracles. The mandibles (fig. 2, *E*) are not much larger than those of the first instar, measuring only 0.06 mm, but they are less heavily sclerotized and have much broader bases. The spiracles of the second thoracic segment are 0.015 mm in diameter. The stumps of rudimentary spiracular branches in the third thoracic and fourth to seventh abdominal segments are more pronounced than in the first instar, and in the head the hypostoma and the postoccipt are more clearly defined. Otherwise the two instars seem to be identical. The internal anatomy is similar to that of *B. compsilurae*, which is shown in figure 5, *A*.

THE THIRD-INSTAR LARVA

Third-instar larvae are from 2.7 mm long and 0.66 mm wide to 3.80 mm long and 1.04 mm wide. The larval form is the same as in the previous instars, but it is distinctly more robust, and the head is wider. The arrangement of the sensory setae is the same as in the first instar. Three pairs were distinguishable on the last abdominal segment, 2 on the dorsal surface and 1 on the ventral. The cuticular spines have practically the same arrangement as before. There are fewest in the dorsal and pleural regions of the segments, and the groups on the posterior portions of the abdominal segments are wider than those on the anterior portions. The last abdominal segment has spines scattered over the dorsal surface, but very few on the ventral surface. The direction of the spines is as before.

The head is like that of the full-grown larva except that it is much smaller and less heavily sclerotized. The tiny spines and sensoria can now be distinguished readily. The mandibles (fig. 2, *F*) are much larger, measuring 0.103 mm, but they closely resemble those of the second instar.

The tracheal system has 9 pairs of open spiracles, 1 pair each on the mesothorax and the metathorax and a pair on each of the first 7 abdominal segments. The mesothoracic spiracle is 0.023 mm in diameter. The eighth abdominal segment has a closed spiracular branch.

The digestive, nervous, and circulatory systems are the same as in the first instar. The mid-intestine, which is clearly visible through the skin, usually exhibits 1 or 2 conspicuous, round vacuoles.

THE FOURTH-INSTAR LARVA

Fourth-instar larvae are from 4.0 mm long and 1.0 mm wide to 6.0 mm long and 1.5 mm wide. They show only minor differences from the preceding instar. The mandibles (fig. 3, *F*) still have the same shape, but they measure 0.15 mm. The mesothoracic spiracles are 0.04 mm in diameter. There are the same number of sensory setae as in the full-grown larva, and their arrangement is the same. The cuticular spines are situated as before, but there are almost none in the pleural and dorsal regions of the first nine segments. The tenth segment has a few in the dorsal region, the eleventh and twelfth segments have them on both dorsal and pleural regions, and the last segment has them on the dorsal surface only.

In the other instars almost all the cuticular spines are straight and have a single point (fig. 3, *C*), although occasionally a spine having

two points is noted. In this instar, however, many of the cuticular spines on the last abdominal segment have several points (fig. 3, *D*).

THE FIFTH-INSTAR LARVA

The full-grown or fifth-instar larva (fig. 3, *A*) has the same general form as the freshly hatched larva. The measurements of individuals range from 6.75 mm long and 2.0 mm wide to 11.5 mm long and 3.0 mm wide. The mesothoracic and metathoracic spiracles and lateral lobes are distinctly lower than the line of spiracles and lateral lobes of the abdominal segments. The mesothoracic spiracles are 0.08 mm in diameter. There are no cuticular spines. The number and arrangement of the sensory setae are indicated in the figure. The first thoracic segment has 6 pairs, the second and third thoracic segments have 5 pairs each, the first 8 abdominal segments have 4 pairs each, the ninth abdominal segment has 3 pairs, and the last abdominal segment has 3 pairs on its dorsal surface and 2 pairs on its ventral surface. The number of sensory setae varies, particularly on the last segment, although most of the specimens examined have the numbers given. Possibly difficulties connected with preparing specimens and locating these minute spines account for the slight variations noted.

The head, seen from the front, presents well-defined characters (fig. 3, *E*). The mandibles (fig. 3, *G*) are 0.22 mm long and 0.19 mm broad. They are very broad and funnel shaped at the base, with heavily sclerotized, reddish brown tips. The inferior pleurostomal rami are connected by a stout crossbar, the hypopharyngeal bracon, extending across the mouth opening just below the mandibles. The transverse bridge of the tentorium is strongly developed, extending across the head between the lateral epicranial angles. There are two distinct dorsal tentorial maculae present on the frons, but no connection between them and the tentorium can be distinguished. Just below each of these marks a tiny oval elevation with a light spot at its center may be noted. These are probably sensoria. The antennae are small nipplelike protuberances surrounded by large oval areas, the antennal foramina. Indistinct sutures lead from the antennal foramina to slight troughlike indentations of the apex of the occiput.

The tracheal system is essentially the same as in the preceding instars, but the branches are much larger and more ramified.

The digestive system is like that of the first instar. Parker (20, fig. 251) has figured the digestive system of *Brachymeria fonscolombei*, which is very similar, although the mid-intestine of *B. intermedia* is less oval and the hind-intestine is much longer and stouter.

The nervous system consists of the brain, the subesophageal ganglia, and the ventral nerve cord. The ventral nerve cord bears 11 pairs of ganglia, which are separated from each other by two heavy fibers. Each ganglion gives off a long nerve fiber, which may transverse several segments. The last ganglion probably represents 2 pairs united, for it gives off 2 pairs of branches. The point of termination of the nerve cord seems to depend on the larval development. In some large prepared specimens it extended to the fourth, and in smaller specimens to the sixth, abdominal segment.

The histoblasts, or imaginal buds, are easily distinguished in this instar. They are white and semiopaque, and lie just beneath the

cuticle. The antennal histoblasts are situated in the antennal rudiments, the leg and wing histoblasts ventrally and laterally in the thoracic segments, and the histoblasts of the genitalia are located ventrally in the eighth and ninth abdominal segments in the female and in the ninth abdominal segment in the male.

The fat body is conspicuous in this instar. It consists of several rows of large oval cells placed between the skin and the integumentary muscles of each segment.

THE PREPUPA

The full-grown larva voids its meconium almost immediately after becoming full fed, and the prepupa may be observed through the loosely fitting larval skin. The prepupa is pure white. At first only the head and thorax can be distinguished, but later the abdomen and appendages of the head and thorax can be differentiated.

THE PUPA

The pupa has a thin, closely fitting, transparent skin, through which the forming adult characters can be clearly distinguished. At first it is yellowish, but as development proceeds pigmentation takes place rapidly and it becomes shiny black.

SEASONAL HISTORY

Brachymeria intermedia overwinters as an adult. Two generations, and possibly a partial third, are completed during the summer months; nevertheless, first-generation females may hibernate successfully.

The adults probably hibernate under the bark of dead trees or in similar places. In the laboratory they remain motionless along the sides or in the corners of the boxes provided for them, but they have never been observed to enter the soil.

At Budapest, Hungary, the insects become active and feed a little during March and April. Attempts were made there to get them to oviposit as soon as possible, in order to determine whether the species went through a generation before attacking *Porthetria dispar*. No eggs were deposited until May 15, when one pupa was apparently attacked, but between May 15 and 25, eggs were deposited in more than 50 preferred-host chrysalids. None of this material, however, produced adults of *B. intermedia*. Seven chrysalids of *Vanessa ranthomelas* were oviposited in on June 7, and from these eggs four *B. intermedia* were reared. Since by this time *P. dispar* pupae are often present in the field in Hungary, it is undoubtedly one of the first species attacked. The first adult *B. intermedia* reared from field-collected *P. dispar* pupae issued on June 28, and issuance continued until July 25. These first-generation adults eagerly attacked a variety of host species during July, August, and September, and second-generation adults began to issue late in August. A second generation is evidently produced under field conditions also, for two *B. intermedia* adults were reared from chrysalids of *Vanessa io* collected at Olaszliszka, Hungary, on August 12, 1926, the adults issuing on September 16 and 20. The second-generation adults that were reared in the laboratory practically refused to oviposit, although they finally did attack some retarded *P. dispar* pupae, and a third generation was reared. It seems questionable, however, if a third generation would develop in the field. Adults of the third generation could not be induced to oviposit.

In New England the seasonal history of *B. intermedia* would probably be somewhat different, for here *Porthetria dispar* completes development much later than it does in central Europe. Pupae are present in the field from about July 5 to August 10, and laboratory rearing indicates that there would be time for *B. intermedia* to complete a generation on other hosts before many *P. dispar* pupae are available. The first host pupae to be parasitized successfully at the Melrose Highlands laboratory were those of *Heterocampa guttivitta* attacked on May 21. A number of host species were attacked during May and the first half of June, and first-generation adults began to issue by June 30 and continued issuing until July 20. It seems probable that these adults would normally attack *P. dispar* pupae available in the field and that second-generation adults would issue during August and September. Whether or not a third generation would develop is questionable. It is known, however, that first-generation adults sent here from Europe attack various American host species in August and September. Therefore, if second-generation adults were issuing from American *P. dispar* at this time, they would probably attack available host species, thus producing a third generation.

HABITS OF THE ADULT

The adult *Brachymeria intermedia* emerges from the host pupa after chewing an irregular hole in the pupal shell. A count of 82 pupae showed 40 exit holes in the anterior end, 37 near the middle, and 5 near the posterior end. Their swollen femora give the adults a clumsy appearance as they slowly move around in confinement, but in nature they are very graceful fliers. Both sexes thrive when confined in glass-topped wooden boxes or cloth-covered cages and fed dry lump sugar and a honey solution (1 part honey to 5 parts water) held on sponges. The cages are kept in a dark, cool place when the insects are not being used. The females live through the winter, but the males die. Difficulty has been experienced in getting the females to hibernate, but most of those that did survive the winter lived well into the summer. A record of 113 hibernated females that issued between July 22 and August 5 of the previous year showed that the first female died on June 18 and the last on August 9, and there was no heavy mortality until the last part of July.

Adults of *B. intermedia* mate readily in confinement. Cloth-covered cages containing several males are placed in the direct sunlight, and a few females are introduced. As the females are mated they are removed, for they mate only once, and the males will continue to pursue them if they are left in the cages. High temperature at midday is the optimum condition, and mating is encouraged if the cage is taken to a shady place after it has been held in the sunlight for about 10 minutes. The time spent in coitu is from 30 to 40 seconds. The age of females seems to be of little importance in mating. Females up to 6 days old mate readily. One female 8 days old was mated in the laboratory, and a few unmated females that hibernated were mated with first-generation males the second summer.

As a rule females do not oviposit readily until 2 or 3 days after mating. Two females were observed to oviposit immediately after fertilization, but no parasites were reared from the eggs. The female crawls onto a host pupa and slowly walks over it a few times. Suddenly she takes a firm grasp on her victim with her powerful hind legs

and, extending her ovipositor, inserts it slowly in the rigid head end of the pupa. The pupa writhes violently, but the parasite shows remarkable ability to hang on as the host rolls around. Oviposition lasts from 40 seconds to 3 minutes. At the end of this time the ovipositor is withdrawn and the host becomes quiet. A small drop of fluid sometimes exudes from the puncture hole, and this the parasite often consumes.

Attempts to determine how many eggs a single female lays were not very satisfactory. At best, oviposition is very slow, and females that were confined individually did not oviposit so frequently as they are believed to do in nature. One female, which laid 48 eggs, was dissected after death, and the ovaries were found to contain a number of well-developed eggs. Apparently they do not lay more than about 6 eggs per day. It was repeatedly noted that 5 females, caged together, would lay 25 or 30 eggs and then show no further interest in host material during that day, and isolated females never laid more than 7 eggs in a single day. An examination of the female reproductive system showed that this is undoubtedly normal. The ovaries have only 3 ovarioles, and, since the eggs are rather large when laid, each ovariole holds only 1 well-developed egg at a time.

Unmated females of *B. intermedia* will oviposit readily, and only males are produced in parthenogenetic reproduction.

No satisfactory records have been kept on the proportion of sexes issuing from field-collected material. In 1930, 2,076 adults were recovered from *Porthetria dispar* pupae collected in Platicevo, Yugoslavia; of these, 682 were males and 1,394 were females. That year, however, the host pupae were held in the field until the first *B. intermedia* adults issued, and probably some males were lost. Experiments in reproduction conducted at the laboratory gave contradictory results. In 1927 mated females that had been reared from *P. dispar* pupae collected in Algeria attacked a number of *P. dispar* pupae on arrival at Melrose Highlands. A total of 228 adult *Brachymeria* were reared, with equal numbers of males and females. In 1930 mated females that had been reared from *P. dispar* pupae in central Europe were used, and 184 adults were reared, but only 26 were males.

TABLE 1.—*Hibernation of Brachymeria intermedia* females in 1930 and 1932

History of material	Box no. ¹	Adults placed in hibernation	Adults successfully hibernating
		Number	Number
First-generation females not used in full reproduction.....	1	50	33
	2	50	23
	3	50	32
	4	60	16
First-generation females used in full reproduction.....	5	50	32
	6	50	27
	7	50	30
	8	38	10
Second-generation females not used in full reproduction.....	9	51	50
	10	30	25
Unmated females not used in full reproduction.....	11	55	12
	12	50	28

¹ Boxes nos. 4, 8, and 11 show hibernation records for 1932; all others show hibernation records for 1930.

So much difficulty was experienced in getting *B. intermedia* adults to hibernate under laboratory conditions that it is believed that there must be rather high mortality in the field. Prior to 1930 so few in-

dividuals lived through the winter that figures are hardly significant. In 1930 and 1932, however, material was held in an unheated insectary in glass-covered wooden boxes measuring 9 by 5 by 3½ inches, and the results were more satisfactory. The records are given in table 1.

The data in table 1 show that first-generation females that oviposit in the fall live through the winter just as well as those that do not oviposit and almost as well as second-generation females. In 1930 the second-generation females hibernated considerably better than those of the first generation, but in 1932 there was little difference. In 1933 it was found that successfully hibernated females that oviposited in the fall of 1932 oviposited almost as well as females that had not previously laid eggs.

DEVELOPMENT OF IMMATURE STAGES

The egg of *Brachymeria intermedia* remains about where it is laid, in the body of the host pupa. It hatches in about 48 hours, and the young larva starts feeding immediately. The larva (fig. 2, A) is remarkably similar to most externally feeding chalcidoid larvae, although it seems to be a true internal feeder.

One of the first activities of the primary larva is the elimination of competitors. Only one parasite ever completes development in the same host pupa, and, although many very young dead larvae have been found in a host, no more than one living well-fed first-instar larva has ever been discovered. Possibly, as in many parasitic Hymenoptera, actual combat between the young larvae is not necessary to cause death, but all dead larvae of this species that have been found bear marks of mandibles, and when living larvae are dissected out of the host pupa and placed together they quickly engage one another.

The young larva develops rapidly and by the end of the second instar has taken up a position on the venter of the host pupa near the junction of the wing covers and the abdominal segments. Here it feeds on the host contents until, by the time it is full grown, most of the anterior portion of the pupa has been eaten out and is occupied by the parasite. *B. intermedia* frequently parasitizes a pupa that contains much more food than is necessary for its development. This surplus remains, gradually drying up. The larva casts its meconium almost immediately after becoming full grown, and prepupal and pupal development follow. The larva usually finishes feeding with its anterior end toward the head of the pupa, and the adults emerge from the anterior end or from the center of the pupal shell.

The time required for development from egg to adult varies greatly with the temperature. *Vanessa urticae* chrysalids attacked on July 31 produced adults of *B. intermedia* in 18 days, but retarded *P. dispar* pupae attacked on September 11 produced *B. intermedia* adults in from 51 to 62 days. A large number of dissections during the normal season of development on *P. dispar* indicate rather clearly, however, that the average time in each stage is as follows: About 2 days in the egg stage, 2 days in each of the first four larval instars, 3 days in the last larval instar, 2 days as a prepupa, and 13 days as a pupa, making a total of 28 days. Females require about 2 days longer for development than males.

FACTORS LIMITING THE EFFECTIVENESS OF THIS PARASITE

There are a number of factors that limit the effectiveness of *Brachymeria intermedia* as a parasite of *Porthetria dispar*. In the

first place, it overwinters as an adult and apparently cannot withstand the rigorous climate that this host endures in central Europe and in New England. It has been extremely important in Sicily and northern Africa, where the winters are mild, but in central Europe it has been difficult to get the parasite to hibernate, and, although it persists in that region and can complete development in a number of hosts, it remains of minor importance. New England has about the same climate as central Europe. *B. intermedia* can develop in a number of American species, but it has never become established in spite of the large colonies of adults that have been liberated. If the gypsy moth or other suitable host were to become established in the southern part of the United States, *B. intermedia* would probably become established there, whereas in New England climatic conditions are unsuitable for its existence.

There must be other factors limiting the effectiveness of *B. intermedia* to account for its sudden disappearance from the forest of Mamora in Morocco, as described by De Lépiney (12). It is difficult, and perhaps unwise, to hazard a theory regarding this phenomenon. It nevertheless seems as though the fact that *B. intermedia* passed through a second generation on some other host might explain this, for, of course, the second-generation host would have to be present in some numbers for the parasite to maintain itself. In any case, the fact that the parasite may require another host species might limit its effectiveness against *P. dispar*.

BRACHYMERIA COMPSILURAE (CWFD.)

DISTRIBUTION AND HOST RELATIONSHIPS

Brachymeria compsiluræ is a North American species which has been taken in northwestern Wisconsin, northeastern Pennsylvania, New Jersey, New York, and all the New England States. Its known range will probably be extended as additional rearing records are obtained.

So far as is known, *B. compsiluræ* acts solely as a parasite of tachinid flies. A list of tachinids from which *B. compsiluræ* has been reared, with their primary lepidopterous hosts, follows. The species that are starred (*) were attacked under laboratory conditions.

TACHINID HOST OF *B. compsiluræ*

Achaetoneura aletiae Riley.
Achaetoneura frenchii Will.
Carcelia laxifrons Vill.

Compsilura concinnata Meig.

Pelatachina pellucida Coq.
Phorocera agilis R. D.
Phorocera claripennis Macq.
Sturmia nidicola Towns.
Sturmia scutellata R. D.
Tachina mella Walk.
Zenillia amplexa Coq.

LEPIDOPTEROUS HOST OF TACHINID FROM WHICH *B. compsiluræ* WAS REARED

Hemerocampa leucostigma S. and A.
Melalopha inclusa Hbn.
Nygmyia phaeorrhoea Don.
(*Porthetria dispar* L.
Nygmyia phaeorrhoea Don.
Stilpnotia salicis L.
{ *Hemerocampa leucostigma* S. and A.
Hamadryas antiopa (L.).
**Bombyx mori* L.
**Euchaetias egle* Dru.
Hamadryas antiopa (L.).
**Porthetria dispar* L.
Hemerocampa leucostigma S. and A.
Nygmyia phaeorrhoea Don.
Porthetria dispar L.
Hemerocampa leucostigma S. and A.
Hemerocampa leucostigma S. and A.

The hosts of *B. compsiluræ* are attacked in the larval stage, while still within the larval skin or pupa of the primary host. The diversity of the lepidopterous hosts indicates that *B. compsiluræ* will oviposit through a wide variety of integuments, and, since it has been found to complete development in so many species of Tachinidae, the actual number of host species which it may parasitize is undoubtedly extremely large.

ECONOMIC IMPORTANCE

Without doubt *Brachymeria compsiluræ* parasitizes large numbers of tachinid parasites, retarding their rate of increase and thus seriously limiting their effectiveness. Two of the most important species that it is known to attack severely are *Compsilura concinnata* and *Sturmia scutellata*. Both these flies have been introduced from Europe in the campaign against the gypsy moth. *C. concinnata* is one of the most effective parasites of the gypsy, brown-tail, and satin moths, and *S. scutellata* is one of the most effective parasites of the gypsy moth. *B. compsiluræ* is therefore of considerable economic importance as affecting the biological control of these pests.

B. compsiluræ attacks its various host species when they are in the larval stage, and the parasite issues from the host puparium. An accurate estimate of the degree of parasitization of any host would therefore have to be based upon an examination of representative samples of puparia collected under field conditions. Unfortunately, however, even when large numbers of Tachinidae are known to be present, it is exceedingly difficult to collect puparia in the soil. A few collections have been made, but most of the records have been taken from puparia formed after the primary lepidopterous host was collected, i. e., when the tachinids were still in the larval stage inside their host. Obviously these tachinid larvae were not exposed to *B. compsiluræ* so long as they would have been under natural conditions, and since *B. compsiluræ* oviposits most readily when the tachinid maggots are full grown, the percentage of parasitization of this stock is probably lower than in material remaining in the field.

Collections made to obtain records on the prevalence of *Brachymeria compsiluræ* fall into four categories. The first consists of a large number of *Sturmia scutellata* puparia obtained over a period of 4 years throughout the area in New England infested with *Porthetria dispar*, in connection with experiments on attractants for male gypsy moths (Collins and Potts, 4). In these experiments female gypsy moth pupae were collected and held on racks until the moths issued. Large numbers of *S. scutellata* maggots issued from these *P. dispar* pupae. Additional puparia were obtained from miscellaneous collections of *P. dispar* material received at the laboratory. The second type of collection consists of several small lots of *S. scutellata* obtained by collecting *P. dispar* pupae at various points. The collections at each point were kept separate in order that the importance of *B. compsiluræ* at widely separated points might be judged. In the third type of collection⁷ *P. dispar* pupae were collected and left in the forest, in order that the *Sturmia* maggots might be exposed to *B. compsiluræ* under field conditions, but that the recovery of puparia might be facilitated by concentration. In spite of these conditions, however, few puparia were found. The fourth type of collection includes four lots of *Compsilura*

⁷ This work was done by T. H. Jones, of the Melrose Highlands laboratory.

concinata puparia that were taken from the field. Table 2 gives the results of these collections in detail.

TABLE 2.—*Brachymeria compsilurae* reared from various lots of tachinid puparia
STURMIA SCUTELLATA

Type of collection	Place collected	Year	Total puparia recovered	Flies	Dead from unknown cause	<i>Brachymeria compsilurae</i> reared	<i>Brachymeria</i> from total puparia
			Number	Number	Number	Number	Percent
(1) <i>Porthetria dispar</i> pupae...	General infested area.	4 years	24, 070	11, 586	10, 546	1, 938	8. 1
	Ossipee, N. H.	1932	879	544	213	122	13. 8
	Francistown, N. H.	1933	494	400	20	74	14. 9
(2) Special collections of <i>P. dispar</i> pupae.	Sebago, Me.	1933	292	213	27	52	17. 8
	Middleboro, Mass.	1933	197	182	7	8	4. 0
	Westford, Mass.	1934	382	221	70	91	23. 8
	Foxboro, Mass.	1934	126	18	64	44	34. 9
	Boxford, Mass.	1927	34	15	17	2	5. 8
	Middleboro, Mass.	1927	96	46	26	24	25. 0
	Boxford, Mass.	1928	31	13	13	5	16. 1
(3) <i>P. dispar</i> pupae left in forest	Saugus, Mass.	1928	120	48	73	8	6. 2
	Nashua, N. H.	1929	16	6	8	2	12. 5
	Boxford, Mass.	1929	10	7	0	3	30. 0
	Dighton, Mass.	1930	149	45	55	49	32. 8
	Middleboro, Mass.	1932	98	45	22	31	31. 6

COMPSILURA CONCINNATA

(4) Field-collected <i>Compsilura concinnata</i> puparia							
(a) In brown-tail moth infestation	Hampton, N. H.	1924	255	40	1 208	3	1. 1
	Marion, Mass.	1933	163	11	1 40	112	68. 7
(b) In gypsy moth infestation	Westford, Mass.	1934	41	11	1 11	19	46. 3
	Cranston, R. I.	1934	432	34	1 144	254	58. 7

¹ Most of dead killed by another parasite.

The record shows considerable variation, with a rather high average abundance of *B. compsilurae*. The most interesting collections are the four lots of field-collected *Compsilura concinnata* puparia (type 4). The lot from Cranston, R. I., was collected a little too late in the season, for between 50 and 60 puparia were found from which adult flies had already emerged. The low percentage of *B. compsilurae* from *C. concinnata* collected in Hampton is probably due to the activities of another secondary parasite, *Monodontomerus aereus* Walk. This species regularly attacks *C. concinnata* puparia in brown-tail moth cocoons, drilling through the puparia with its ovipositor. Laboratory experiments show that it will develop just as readily on *B. compsilurae* as on *C. concinnata*. The year that this collection was made *M. aereus* was very abundant, killing almost all the *Compsilura*.

The number of *B. compsilurae* reared from *Sturmia* puparia that were dug up after exposure in the forest (type 3) should represent nearly natural conditions. Considerable variation in the degree of parasitization is noted, and in some lots it was very high.

The large general collections (type 1) represent such a representative sample that if the tachinid larvae had been subjected to the same exposure to attack as under normal field conditions the figures would be extremely valuable. Nevertheless, they are useful in showing the approximate conditions.

The small special collections (type 2) are valuable because they show that *B. compsilurae* is present over the whole area.

THE ADULT

(*Chalcis*) *Brachymeria compsiluræ* was described by Crawford (6) in 1911. The adult is similar to *B. intermedia* (fig. 1, *A*), although the markings of the hind legs and the malar carinae show differences, as illustrated in figure 1, *D* and *E*.

THE EGG

The egg (fig. 4, *B*) is of the usual form, but is unusual in being surrounded by a clear, hyaline membrane. The egg body averages

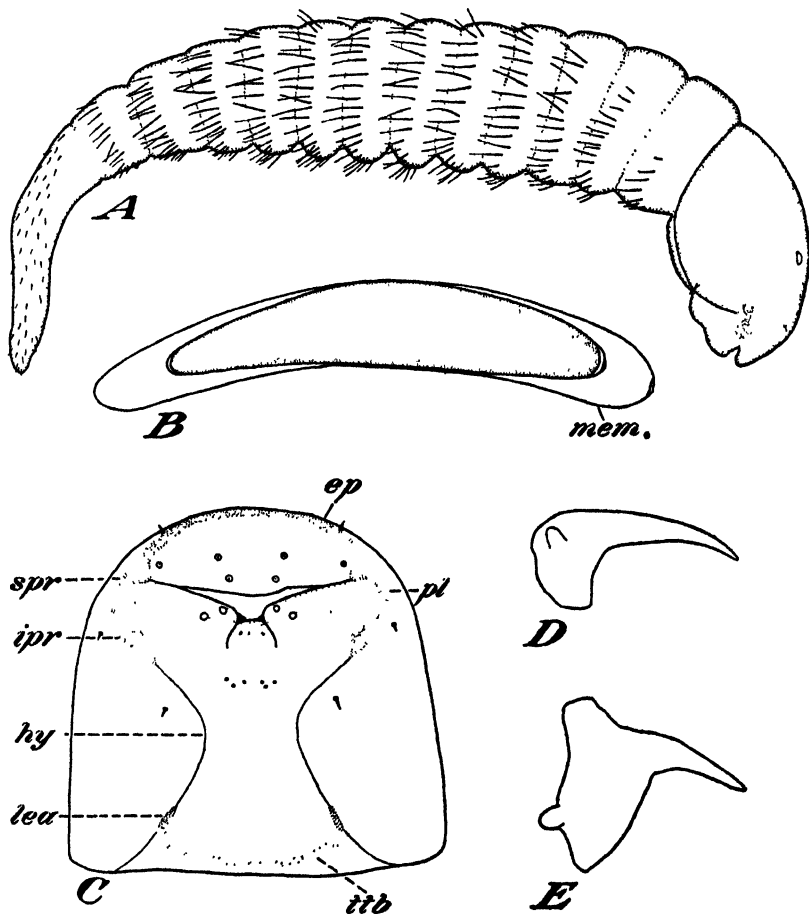


FIGURE 4.—*Brachymeria compsiluræ*: *A*, First-instar larva (×120). *B*, Egg, showing membrane, *mem* (×55). *C*, Head of first-instar larva (×275); *ep*, epistoma; *spr*, superior pleurostomal ramus; *plr*, pleurostoma; *ipr*, inferior pleurostomal ramus; *hy*, hypostoma; *lea*, lateral epicranial angle; *ttb*, transverse tentorial bar. *D*, first-instar mandible (×400). *E*, Second-instar mandible (×500).

1.04 mm long and 0.23 mm wide. It is slightly curved, with rounded ends, being somewhat wider at the cephalic end. The chorion is smooth except for a slightly roughened area at the micropyle. The embryo is yellowish. The envelope is about the same shape and width as the egg body, but it is considerably longer. It is so delicate

that it collapses when the egg is dissected out of the host, but soon regains its shape if floated in water. When floated in normal saline solution, the membrane becomes slightly turgid. An average specimen measures 1.60 mm long and 0.23 mm wide, while some specimens as long as 2.00 mm have been noted. The cephalic end bears a slightly roughened area comparable to the micropyle of the egg body.

THE FIRST-INSTAR LARVA

The first-instar larva (fig. 4, *A*) differs from that of *Brachymeria intermedia* in having a well-developed tail. It closely resembles that of *B. fonscolombei*, belonging to Parker's group 5 (20, fig. 104).

The newly hatched larva is 1.21 mm long and 0.18 mm wide; its body form is shown in figure 4, *A*. The head is well differentiated, followed by 13 distinct segments, the last of which is prolonged into a tail. The tail is as long as 3 or 4 body segments and tapers posteriorly. Parker (20, p. 270) found that, contrary to the usual manner in larvae, the tail of *B. fonscolombei* probably results from a fusion of body segments 12 and 13, there being only 12 segments in his larva. *B. compsilurae*, on the contrary, has 12 distinct segments followed by the tail. Each of the body segments bears a transverse row of long delicate spines, as indicated in the figure. The spines on the tail are considerably shorter and broader than the others. No sensory setae were distinguished on the body segments.

The head is thimble shaped and lightly sclerotized. The antennae are inconspicuous rounded elevations. The mouth opening is on the ventral side at the apex. The mandibles (fig. 4, *D*) are 0.066 mm long. They are well sclerotized and yellowish at their tips. Viewed ventrally, the head (fig. 4, *C*) presents well-defined characters, although the sensoria are distinguished with difficulty and those on the labium have been seen only in living specimens. The weekly sclerotized hypopharyngeal bracon is not indicated in the figure. It is strongly depressed and weak at the center.

The tracheal system is composed of a pair of lateral longitudinal trunks united anteriorly in the first thoracic and posteriorly in the ninth abdominal segment. There are no open spiracles, but rudimentary spiracular branches occur in the second and third thoracic and the first seven abdominal segments. The dorsal tracheal branches arise from both the spiracular branches and the main longitudinal trunk. The ventral branches arise from the longitudinal trunk near the base of the spiracular branches. The head is supplied from branches arising in the first thoracic segment, and the tail receives long branches from the longitudinal trunks in the ninth abdominal segment.

The digestive, nervous, and circulatory systems are similar to those described for *Brachymeria intermedia*, with the following minor exceptions: The hind-intestine terminates on the ventral surface of the basal part of the tail, the salivary glands are somewhat less regularly serpentine and they terminate in the eighth abdominal segment, the dorsal malpighian tubes are not sinuous but straight, the anterior malpighian tubes extend forward to the first or second abdominal segment, and the ventral nerve cord extends back to the eighth abdominal segment, where the last fused ganglion gives rise to 3 pairs of nerve cords instead of 2.

THE SECOND-INSTAR LARVA

Second-instar larvae are from 1.58 mm long and 0.44 mm wide to 2.28 mm long and 0.75 mm wide. In its form this instar (fig. 5, *A*) is

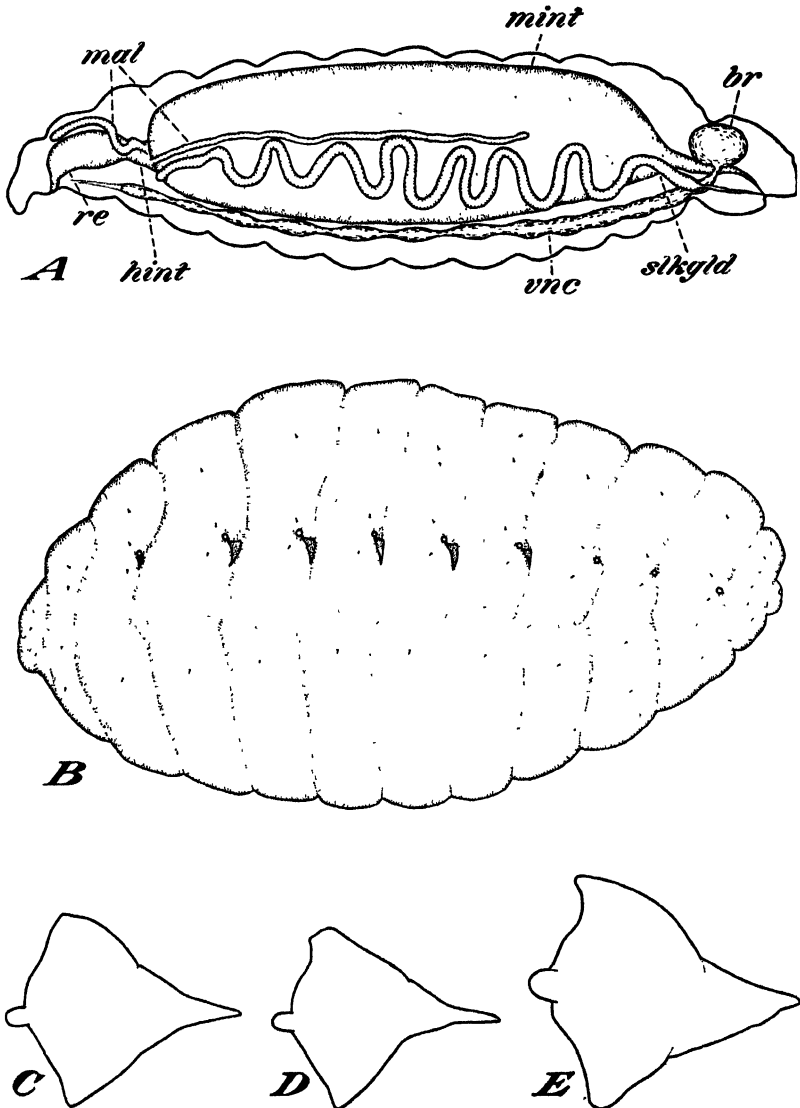


FIGURE 5.—*Brachymeria compsiturae*: *A*, Second-instar larva, internal anatomy ($\times 62$); *br*, brain; *mint*, mid-intestine; *mal*, malpighian tubes; *re*, rectum; *hint*, hind-intestine; *vnc*, ventral nerve cord; *slkgld*, silk gland. *B*, Full-grown larva ($\times 10$). *C*, Third-instar mandible ($\times 387$). *D*, Fourth-instar mandible ($\times 234$). *E*, Fifth-instar mandible ($\times 200$).

quite different from the first instar. The body is much stouter and the tail, although still pointed, is much shorter and thicker. The cuticular armature is also entirely different in this instar. The spines are very short, and each segment bears several rows. The first

segment has 3 or 4 rows on the venter which extend about to the median line of the pleurum, but it has none on the dorsum. All the other segments have 3 or 4 rows on the venter which gradually disappear toward the median line and then increase until 2 or 3 rows of somewhat shorter spines are present on the dorsum. The last 3 abdominal segments have more spines on the dorsum than on the venter, and the tail is thickly covered on the dorsum but is bare on the venter. All the cuticular spines are pointed backwards. No sensory setae were distinguished on the body segments.

The head, although more heavily sclerotized, has the same characters as in the first instar. The antennae are much more prominent. The mandibles (fig. 4, *E*) are much broader at the base, but are actually shorter, being only 0.053 mm long.

The tracheal system, as in the first instar, has no open spiracles, but rudimentary spiracular branches may be distinguished in the second and third thoracic and first 7 abdominal segments.

THE THIRD-INSTAR LARVA

Third-instar larvae are from 2.73 mm long and 1.10 mm wide to 3.52 mm long and 1.33 mm wide. The body is stouter than in the second instar. The tail is still noticeable, being pointed, but it is very little longer than the other body segments. The cuticular armature is almost the same as in the second instar, but usually there are a few forward-pointed spines on the posterior ventral borders of the first 6 or 8 abdominal segments. In this instar sensory setae were distinguished on the body segments for the first time. Each segment except the last apparently has 3 pairs, 1 on the dorsum, 1 near the spiracles, and 1 half way between the spiracles and the venter. None was found on the last abdominal segment.

The head is much smaller, but otherwise exactly like that of the full-grown larva. The mandibles (fig. 5, *C*) are 0.086 mm long.

The tracheal system has 9 pairs of open spiracles, 1 pair on each of the second and third thoracic and on each of the first 7 abdominal segments. The mesothoracic spiracle is approximately 0.023 mm in diameter.

THE FOURTH-INSTAR LARVA

Fourth-instar larvae are from 4.42 mm long and 1.76 mm wide to 5.90 mm long and 2.64 mm wide. The body is well rounded, approaching the appearance of the full-grown larva. The cuticular armature has the same arrangement as before, although there is a slight increase in the number of rows of spines on each segment. The number and arrangement of the sensory setae is exactly the same as in the full-grown larva (fig. 5, *B*).

The head has the same characters as the full-grown larva. The mandibles (fig. 5, *D*) are about 0.12 mm long.

The tracheal system is like that of the third-instar larva. The mesothoracic spiracles are about 0.04 mm in diameter.

THE FIFTH-INSTAR LARVA

When reared from the large tachinid, *Sturmia scutellata*, the full-grown, or fifth-instar, larva (fig. 5, *B*) is from 5.7 mm long and 2.6 mm wide to 10.0 mm long and 5.0 mm wide, but when reared from *Comp-*

silura concinnata, which is a much smaller tachinid, the full-grown larva averages about 5.72 mm long and 2.82 mm wide. It is strikingly oval in shape and of a glistening yellowish-white color with distinct segmentation. The tail is simply a tiny terminal segment, somewhat pointed. There are no cuticular spines. The sensory setae are inconspicuous, the largest being only 0.033 mm long, but their position has been indicated in the drawing. Their number and position are the same as in the full-grown larva of *B. intermedia* (fig. 3, A). Little wedge-shaped, yellowish, sclerotized plates are conspicuous just below the spiracles of abdominal segments 2 to 7, inclusive. Parker (20, pp. 298-299) has also described such plates in *B. fonscolombei*. Their function is unknown. On freshly molted fifth-instar larvae these plates cannot be distinguished. They rapidly become yellowish, however.

The head is so much like that of *B. intermedia* that it will not be described or illustrated separately. The only apparent differences are as follows: The 2 pairs of sensory spines that lie above and laterad of the antennae in *B. intermedia* cannot be distinguished in *B. compsiluræ*; the antennae of *B. intermedia* are simple, while in *B. compsiluræ* they are almost invariably divided or two-segmented; finally, the mandibles of *B. intermedia* (fig. 3, G) are less robust than those of *B. compsiluræ* (fig. 5, E). The mandibles of *B. compsiluræ* are about 0.17 mm long and 0.15 mm wide.

The tracheal system is essentially the same as in the preceding instars, but the branches are much larger and more ramified.

The digestive system is practically the same as in the second instar (fig. 5, A), although the mid-intestine is now a large oval sac. In the full-grown larva the salivary-gland tubes extend only to the sixth abdominal segment. Parker's figure (20, fig. 251) shows the digestive system of *B. fonscolombei* to be very similar, although the hind-intestine in *B. compsiluræ* is much larger, occupying a large part of the ninth abdominal segment.

The nervous system is of the usual type, consisting of the brain, subesophageal ganglia, and nerve cord connecting 11 pairs of ganglia. Each ganglion gives off a long nerve fiber, and the terminal pair probably represents the fusion of 3 pairs, because 3 pairs of nerve fibers arise from it. The terminal ganglion is in the sixth abdominal segment.

The histoblasts and the fat body are conspicuous in this instar, occupying the same relative positions as in the full-grown larva of *B. intermedia*.

THE PREPUPA

If a second generation of *B. compsiluræ* develops during the season, the fifth-instar larva voids its meconium almost immediately after becoming full fed, and the prepupa may be clearly distinguished, as in *B. intermedia*. If another generation of *B. compsiluræ* does not develop during the season, the winter is passed as a full-grown larva, and the meconium is voided in the spring.

THE PUPA

The pupa is of the usual hymenopterous type, presenting no great differences from that of *B. intermedia*.

SEASONAL HISTORY

Brachymeria compsilurae overwinters as a full-grown larva within the puparium of its host, and its seasonal development depends to a large extent upon the host which it parasitizes. If the adult attacks a single-generation tachinid, such as *Sturmia scutellata*, the resulting progeny remain as full-grown larvae until the following year, thus completing but one generation. But if the adult attacks a multi-brooded tachinid, such as *Compsilura concinnata*, 1, 2, or even 3 generations may develop during the season. These conditions can be described best by comparing the life history of the parasite upon the two different types of tachinid host species, but it must be borne in mind that in nature the parasite may readily shift from one to the other.

When *B. compsilurae* parasitizes *S. scutellata*, adult emergence begins about the middle of June and continues until about the middle of July. *P. dispar* pupae are already present in the field at this time, and *Sturmia* maggots may be attacked from about the middle of July until the middle of August. *B. compsilurae* larvae rapidly become full grown, and hibernation takes place in this stage. The meconium is cast the next spring, and prepupal and pupal transformations follow in short order.

When *B. compsilurae* parasitizes *Compsilura concinnata*, adult emergence begins about June 1 and continues until about June 20. The first hosts attacked in the field are probably *C. concinnata* developing in larvae of *Stilpnotia salicis* or *Porthetria dispar*, which become available about June 20. Second-generation adults of *Brachymeria* begin issuing about July 21 and continue to come out until the first of August. In the laboratory these second-generation adults attacked *C. concinnata* developing in *Hyphantria cunea* Dru. and *Euchaetias egle* Dru. between July 29 and August 12. Third-generation adults appeared between August 28 and September 8. Females of this generation attacked *C. concinnata* developing in *E. egle* between September 6 and 13. All the full-grown larvae of this generation hibernated.

TABLE 3.—*Summer-issuing and hibernating Brachymeria compsilurae* from *Compsilura concinnata* attacked on different dates in 1933

Generation of adult parasite that oviposited	Date of attack	Summer-issuing <i>Brachymeria</i>	Hibernating <i>Brachymeria</i>
		Number	Number
First:			
Adult issued from <i>Sturmia scutellata</i>	June 24.....	9	1
	June 25.....	19	
	June 26.....	29	10
Adult issued from <i>Compsilura concinnata</i>	June 27.....	6	17
	June 28.....	42	0
Second.....	July 29.....	6	0
	Aug. 5.....	3	10
	Sept. 6.....	0	17
	Sept. 7.....	0	9
Third.....	Aug. 11.....	0	5
	Aug. 13.....	0	2

This summary of the development of *B. compsilurae* in *C. concinnata* does not take into consideration the number of larvae of each generation which hibernate. This phase in the life history is confusing, for

there does not seem to be any rule governing the diapause. In 1932 the number of larvae entering hibernation seemed to increase as the season advanced. From *C. concinnata* attacked by first-generation adults from June 29 to July 1, 49 *Brachymeria* were reared; 39 adults issued the same season, while 10 larvae, or about 20 percent, hibernated. Out of 95 *Brachymeria* reared as progeny of second-generation adults which attacked *C. concinnata* from August 6 to 18, 20 adults issued, and 75 larvae, or about 80 percent, hibernated. In 1933, however, there seemed to be no definite hibernation tendency. In one lot attacked on June 26 the majority of *B. compsilurae* issued the same season, while in other lots attacked on the same date the majority hibernated. The results are shown in table 3.

These records show not only the extreme variability in hibernation tendency in the first two generations reared from *Compsilura*, but also that, whether the first-generation adults issue from *Sturmia* or from *Compsilura*, they readily attack *Compsilura* and their progeny exhibit the same tendencies regarding summer issuance.

HABITS OF THE ADULT

When *Brachymeria compsilurae* is ready to emerge, it first cuts a small hole at about the third segment of the host puparium. It then cuts around the puparium, roughly following the line of cleavage between the cap and the rest of the puparium, by placing one mandible inside and one outside and chipping away at the hard shell. When the cut extends about four-fifths of the way around, the parasite pushes the cap off with its head. The adults, like those of *B. intermedia*, appear very clumsy in confinement, but they are graceful fliers. Both sexes thrive in confinement when held in cages like those used for *B. intermedia*. Ten females that issued on June 15, and were used almost daily in reproduction work, lived an average of 40 days, with a minimum of 27 and a maximum of 57 days. Twenty males issuing on June 13 lived an average of 49 days, with a minimum of 17 and a maximum of 81 days. If the insects are kept continuously in a cool, dark place, they will, of course, live much longer. One male held in this manner lived 121 days and one female lived 140 days.

Brachymeria compsilurae adults mate readily in confinement, mating proceeds almost exactly as it does with *B. intermedia*, and the same optimum conditions hold for both species. Pairs remained in coitu between 10 and 20 seconds, with an average of 15½ seconds, in 12 recorded instances. The age of the female seems to have little influence on fertilization. Three females that were 20 days old and had already laid a number of unfertilized eggs mated readily. They subsequently oviposited again, and both sexes were represented in their progeny, indicating that they had been fertilized.

Some *B. compsilurae* females have been known to oviposit within a day after emergence, but oviposition does not proceed readily until they are 2 or 3 days old. Interest is shown in any lepidopterous larva or pupa that is dead or motionless, but seldom is much attention paid to a primary host that does not contain a tachinid maggot. Living larvae or pupae are avoided. After the female has chosen her victim, she mounts it and walks over it slowly. Now and then she stops, elevates her body, unsheaths her ovipositor, and slowly pushes

it into the caterpillar or pupa. If she encounters a tachinid maggot, the female oviposits immediately. If, however, she finds none, she moves forward, withdrawing her ovipositor. Usually a female inserts her ovipositor in a caterpillar or pupa several times and is often occupied with it for 10 minutes or longer. If other females are present, they often strike at one another with their hind legs, but these battles are seldom serious enough under laboratory conditions to interfere with the more imperative desire to oviposit, and often 2 or 3 females will oviposit in 1 maggot at the same time.

Evidently a tachinid maggot itself is not enough to induce oviposition, for free maggots are avoided. The dead or dying primary host must be present, and there is evidence that the parasites are guided somewhat by the sense of smell. Several times, when an ovipositing female caused a maggot to writhe with especial violence, a drop or two of the brown liquid commonly surrounding an almost full-fed tachinid maggot oozed out on the vial or box. Other females would repeatedly try to insert their ovipositors in these drops of liquid. As soon as they were dry, however, no further attention was paid them.

The reproductive capacity of the females was not determined satisfactorily. Ten females were isolated and kept constantly supplied with host material, but they did not live long. Six of them laid from 38 to 48 eggs each, with an average of 42, but upon dissection large numbers of eggs were still present in the ovarioles. No more than 12 eggs were laid in a single day, but this number was frequently obtained. This is probably the total daily capacity, for each ovary has 6 ovarioles, and the eggs are apparently too large to permit development of more than 1 at a time.

Unmated *B. compsiluræ* females oviposit readily, and only males are produced by parthenogenesis. No satisfactory records have been kept on the proportion of the two sexes issuing from field-collected material, but there are indications that the sexes are about equal. In 1932 a lot of 70 individuals comprised 29 males and 41 females, and in 1933 a lot of 100 individuals consisted of 57 males and 43 females.

DEVELOPMENT OF IMMATURE STAGES

The egg of *Brachymeria compsiluræ* remains about where it is laid, in the body cavity of the host maggot. Hatching takes place in about 48 hours, and the young larva starts feeding immediately on the blood and lymph of the host. The parasite larva is a typical internal feeder, having a long tail and a closed tracheal system. It closely resembles the larva of *B. fonscolombei*, which was figured by Parker (20, figs. 103-104). No more than one *B. compsiluræ* larva ever completes development in a host, even when more than one egg is laid. As with *B. intermedia*, all the dead larvae observed have borne marks that might have been made by the mandibles of another larva. The young larva apparently does not develop beyond the first instar until the host metamorphosis from larva to pupa takes place. It then apparently feeds indiscriminately, for it soon eats out a bubblelike area in the host's body contents, and by the time it is in the second instar it can usually be found in this space. The larva invariably consumes the entire body contents of the host, which vary greatly in size, and there is therefore considerable varia-

tion in the size of the adult parasites. When feeding is completed, the larva may void its meconium and transform to a pupa, or it may remain as a larva in the host puparium all winter, voiding its meconium in the spring. When feeding is completed, the head end of the parasite is always directed toward the cephalic end of the puparium.

The time required for development from egg to adult depends somewhat on the condition of the host larva when it is attacked. If the tachinid larva is full grown and issues from its host within 48 hours after the *Brachymeria* egg is laid, the hyperparasite develops rapidly. If the tachinid larva does not issue and form its puparium for several days, growth of the hyperparasite larva is retarded. It apparently never develops beyond the first instar until the host forms its puparium. A first-instar parasite larva has been found in a tachinid maggot 6 days after egg deposition. If the maggot had formed its puparium on the first or second day, the larva would probably have been in the third instar. Temperature also affects the rate of development. Under laboratory conditions host larvae attacked during June, July, and August showed no great variations, however. From 23 to 28 days were required for the development of males and from 25 to 31 days for that of females. Larval development was observed during July by dissecting 57 host puparia that were formed 2 days after the maggots were attacked. These dissections indicated that 2 days were spent as an egg, about 2 days as a first-instar larva, 1 day in each of the second and third larval instars, 2 days in the fourth instar, 3 days in the fifth instar, 2 days as a prepupa, and 12 days as a pupa, making a total of approximately 25 days.

FACTORS LIMITING THE EFFECTIVENESS OF THIS PARASITE

Brachymeria compsiluræ is a species that has taken advantage of a new supply of food and has increased enormously in numbers. Its principal host species, *Compsilura concinnata* and *Sturmia scutellata*, have been established in North America so long, however, that, if the secondary parasite were capable of overtaking them, it would probably have done so by this time. Just what has limited its increase is problematical. The only known factor curtailing it is another secondary parasite, *Monodontomerus aereus* Walk., which undoubtedly parasitizes *B. compsiluræ* when it is a parasite of *C. concinnata* in *Nygmia phaeorrhoea*. The number that are thus accidentally attacked must be very small, however. Possibly the wide range of hosts in which *C. concinnata* develops protects this species somewhat, but there seems to be nothing to protect *S. scutellata*. The fact remains that, in spite of serious losses through *B. compsiluræ*, both these beneficial tachinids have continued to maintain themselves.

COMPARISON OF THE TWO SPECIES

The adults of *Brachymeria intermedia* and *B. compsiluræ* have been found to be very similar. Their habits of mating are almost identical, their methods of oviposition are much the same although the stage and condition of the host oviposited in are different, and their seasonal histories are similar, with each producing several generations. But here the similarity ends. The two species present entirely different

host relations, their hibernation habit differs, and their immature stages are radically different.

The egg of *B. intermedia* is of the conventional type, whereas that of *B. compsilurae* is unusual in that it has an enveloping membrane. No other hymenopterous egg with such a membrane has been figured, although the writer has found one around the egg of *Cremastus interruptor* Grav., a parasite of *Rhyacionia buoliana* Schiff. The first-instar larva of *B. intermedia* closely resembles external feeding larvae, but that of *B. compsilurae* is typical of the caudate form of internal feeding chalcidoid larvae. The advanced instars of *B. intermedia* do not differ greatly from the first instar, but in the case of *B. compsilurae* the appearance changes considerably owing to the gradual loss of the tail, and the full-grown larva bears 6 sclerotic plates below the spiracles of abdominal segments 2 to 7, inclusive. The head of the full-grown larva, however, is practically the same in both species, and their internal anatomy is very similar.

SUMMARY

Brachymeria intermedia (Nees) and *B. compsilurae* (Cwfd.) are, respectively, primary and secondary parasites of the gypsy moth (*Porthetria dispar* L.).

Brachymeria intermedia is a polyphagous European species which is of primary importance as a parasite of *P. dispar* pupae in Sicily, Algeria, and Morocco. It is a minor factor in the more northern latitudes of central Europe, probably requiring a warmer climate to become effective. Several unsuccessful attempts have been made to establish it in New England. This species overwinters as an adult, passing through 2, and possibly 3, generations in a season. *P. dispar* is probably the first host species to be attacked in central Europe after the adults leave hibernation. The adults thrive and mate readily in confinement. They oviposit in the host pupa, and development from egg to adult requires from 18 days in August to 62 days for eggs laid in September. The egg of *B. intermedia* is of the conventional type. There are five larval instars. The first-instar larva closely resembles typical external feeding chalcidoid larvae, and the advanced larval instars do not differ greatly from the first instar.

Brachymeria compsilurae is a species occurring in North America which, so far as is known, acts solely as a parasite of tachinid flies. It has been reared from many species of Tachinidae, and is particularly destructive to *Compsilura concinnata* and *Sturmia scutellata*, which are European parasites successfully established in New England against the gypsy moth. *B. compsilurae* overwinters as a full-grown larva within its host puparium. It develops only 1 generation in single-generation tachinids, but passes through 2 or 3 generations in multi-brooded host species. The adults thrive and mate readily in confinement. They oviposit through the primary lepidopterous host pupa or larva, locating the tachinid maggots probably through a sense of smell. Development from egg to adult requires from 23 to 28 days in males and from 25 to 31 days in females. The egg is unusual in that a clear hyaline membrane encloses an egg body of the conventional type. There are five larval instars. The first-instar larva is typical of the caudate form of internal feeding chalcidoid larvae. The advanced instars change considerably owing to the

gradual loss of the tail. The full-grown larva is unusual in having 6 sclerotic plates below the spiracles of abdominal segments 2 to 7, inclusive.

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TRICHOSPORIUM SYMBIOTICUM, N. SP., A WOOD-STAINING FUNGUS ASSOCIATED WITH SCOLYTUS VENTRALIS¹

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INTRODUCTION

During the past several years an engraver beetle (*Scolytus ventralis* Lec.) has caused the death of many white firs (*Abies concolor* Lindl. and Gord.) throughout the Sierra Nevadas of California. The galleries are confined to the cambial region, and the eventual death of the infested tree is the result of girdling.

A brown discoloration has been known to be commonly associated with *Scolytus ventralis* tunnels, but it was not until 1930 that the cause of the stain was discovered. In that year an investigation was started by the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, on the insects infesting white firs, and Struble³ isolated a fungus from a number of the stained areas. Inoculations with the isolated organism produced a similar brown discoloration in the wood and inner bark of uninfested trees.

Subsequent to this work and at the invitation of the Division of Forest Insects, the writer carried on additional studies of the fungus in an attempt to determine its role in the development of beetle broods. The results of these studies and a description of the fungus are given in the present paper. Special effort has been made to determine the pathogenicity of the fungus to white fir and the manner in which it is transmitted from tree to tree.

REVIEW OF LITERATURE

In 1931 Rumbold (13)⁴ reviewed the literature relating to bark beetles and blue-staining fungi. In her own investigations she established the fact that blue stains are frequently associated with species of *Ips* and *Dendroctonus*. Grosmann (7) studied *Ipids* and concluded that the different associated fungi have the same requirements for living as the beetles but that the insects are important carriers of blue stain and related organisms. Investigators have shown that bark beetles frequently act as direct carriers of infection.

Ceratostomella (Graphium) ulmi (Schwarz) Buisman, which causes the serious Dutch elm disease, seems to be the only wood-staining fungus so far reported to be associated with *Scolytus* beetles. Wollenweber

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² Acknowledgment is made of the helpful cooperation of the personnel of the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. An expression of appreciation is especially due George R. Struble, of that Division. Two illustrations (figs. 4 and 5) were supplied through the courtesy of the same Division. In the determination of the specific fungus the writer received valuable assistance from members of the Division of Forest Pathology, Bureau of Plant Industry, especially from C. Audrey Richards, Caroline T. Rumbold, and Ross W. Davidson. Dr. Lee Bonar, associate professor of botany, University of California, gave valuable help in the allocation of the species.

³ STRUBLE, G. R. THE FIR ENGRAVER BEETLE AND ASSOCIATED INSECTS IN WHITE FIR. PRELIMINARY REPORT. U. S. Dept. Agr., Bur. Ent. 1931. (Unpublished.)

⁴ Reference is made by number (italic) to Literature Cited, p. 538.

and Stapp (14) suggested that the frequent occurrence of *G. ulmi* in connection with *Scolytus* galleries may indicate that these beetles aid in spreading the disease, although actual proof was not obtained. Betrem and collaborators (3) showed, however, by isolations that *S. scolytus* Fab. adults actually carry the spores of *G. ulmi* both internally and externally. Since the spread of the Dutch elm disease to the United States, Beattie (1) has reported that May and Fowler isolated *G. ulmi* from imported elm logs containing *S. scolytus* broods. This interception was later supplemented by others and strongly suggests that these beetles may have played an important part in introducing the disease from abroad.

In some instances a symbiotic relationship between fungi and beetles has been indicated. Hubbard (9) in his study of ambrosia beetles showed that an interdependence does exist. For bark beetles true symbiosis is unusual.

MATERIALS AND METHODS

In order to secure an abundant supply of recently infested white fir material and newly emerged beetles, field laboratories were established in close proximity to the forest entomology camps where studies of *Scolytus ventralis* were at the time being pursued. The camps were situated at different localities, at elevations of 3,000 to 5,000 feet. Occasional material collected from other areas was also utilized in the course of the study.

The investigation so far has been confined to white fir, although *Scolytus ventralis* also infests other species, such as red fir (*Abies magnifica* Murr.), in this same section. Since the latter species occurs at higher elevations, the beetle emergence period is considerably shorter and infestations are less favorable for continued study.

Material for a close study of the fungus that causes the stain was obtained from freshly infested firs. Individually stained areas of xylem and inner bark were prepared for culturing by cutting away surface contamination with a flamed scalpel, thereby exposing fresh stain. Slivers were removed aseptically and planted on malt agar (2.5 percent malt extract to 2.5 percent agar) in Petri dishes. Isolations were later transferred to test-tube slants.

THE STAINING FUNGUS

ISOLATION

Stained material was cultured from 100 different galleries during the course of the study. A summary of the isolation results is presented in table 1.

TABLE 1.—Isolations from *Scolytus ventralis* galleries in *Abies concolor*

Material	Location of tree	Total galleries cultured	Cultures from galleries showing --		
			Causal organism	Other fungi	No organism
		Number	Number	Number	Number
Recent stain in inner bark	Stanislaus National Forest ..	16	14		2
Recent stain in xylem	do	10	8		
1-year-old stain in xylem	do	11	6	2	2
Recent stain in inner bark	Sequoia National Forest ..	8	8		
Recent stain in xylem	do	32	26	4	2
do	do	13	10	2	1
Recent beetle entrance holes	Sierra National Forest ..	10	5	5	
Total		100	77	16	7

From a total of 100 isolations attempted, 77 produced cultures of the causal organism, 16 contained other fungi, and 7 were negative. The fungi other than the causal organism did not occur consistently. A few of the more unusual types were reduced to pure cultures for use in future tests to determine their staining possibilities.

In a few cases conidiophores of the fungus were observed as delicate, whitish hyphae in vacated *Scolytus ventralis* galleries. Because of their fragile character it is difficult to detect such formations regularly, and cultures were needed for verification.

Unlike the common blue stains in trees, the brown discoloration does not penetrate deeply into the sapwood and is confined to the annual ring in which the galleries are made.

A comparison of the fungus with Struble's isolations showed that the strains were identical in cultural as well as in microscopic features.

IDENTITY OF THE FUNGUS

There is general agreement that the fungus belongs in the Dematiaceae and to the genus *Trichosporium* Fr. No description has been found in the literature which can be applied to the organism referred to in this paper. It therefore appears necessary to designate the fungus as a new species.

TECHNICAL DESCRIPTION

Trichosporium symbioticum, n. sp.

Hyphis septatis, pallidis vel fuscis brunneis, intercellularibus, mediocribus 3.0μ diam.; conidiophoris ramosis, septatis, hyalinis, 1μ – 1.5μ crassitudine; conidiis hyalinis, subgloboosis, sessilibus, apice insertis, capitulatis et subterminalibus pleuraeogenis, 1.8μ – 2.4μ diam.

Hyphae septate, colorless to brown, intercellular, averaging about 3μ in diameter. Conidiophores septate, colorless, 1μ – 1.5μ in diameter, and bear clusters of spores inserted on the apices or occasionally subterminally of the main axis or on secondary or tertiary branches. Spores attached at the tips, hyaline, subglobose, 1.8μ – 2.4μ in diameter. Associated with *Scolytus ventralis* Lec. galleries, causing a brown stain in the cambium and adjacent xylem and phloem of *Abies concolor* Lindl. and Gord. in California.

In malt-agar cultures, submerged mycelium eventually fuscous brown, averaging 6μ in diameter, conidiophores colorless, clustered, erect, septate, 1.5μ – 2μ thick, spores variable in size, 1.5μ – 4μ in diameter.

On Abietineae (*Abies concolor* Lindl. and Gord.), California.

Type locality.—Strawberry, Stanislaus National Forest. Stan. W-201 wood and bark, herbarium, Division of Forest Pathology, San Francisco, Calif. Parts of type also deposited in mycological collections, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture. Test-tube and plate cultures deposited with each collection.

The two distinguishing features that segregate this species from most other described species of *Trichosporium* are the colorless spores and their minute size. Descriptions of species that have colorless spores were found inapplicable, mainly because of their considerably larger spore size or in some cases because other characters as well did not agree, such as the color of the hyphae and the shape of the spores. A few of the descriptions given in the literature were not adequate for accurate comparison.

CULTURAL CHARACTERISTICS

Pure cultures of the fungus were grown on malt, potato-dextrose, corn-meal, starch, and modified Czapek's⁵ agar. On malt agar

⁵ KH_2PO_4 was used in place of K_2HPO_4 since an acid medium is more satisfactory for growing wood-inhabiting fungi.

which was the most favorable medium used, two kinds of mycelium were produced, (1) a white flocculent aerial mycelium, bearing conidiophores and spores, and (2) dark submerged hyphae, varying from cinnamon drab to fuscous brown in color. The submerged hyphae do not show noticeable coloration until the culture is 10 to 15 days old. The older the culture the darker the submerged mycelium becomes. On other agars the mycelium shows different degrees of coloration, all less distinct than that on malt agar, and no coloration on the Czapek's agar. Figure 1 shows the characteristic development of the mycelium under cultural conditions.

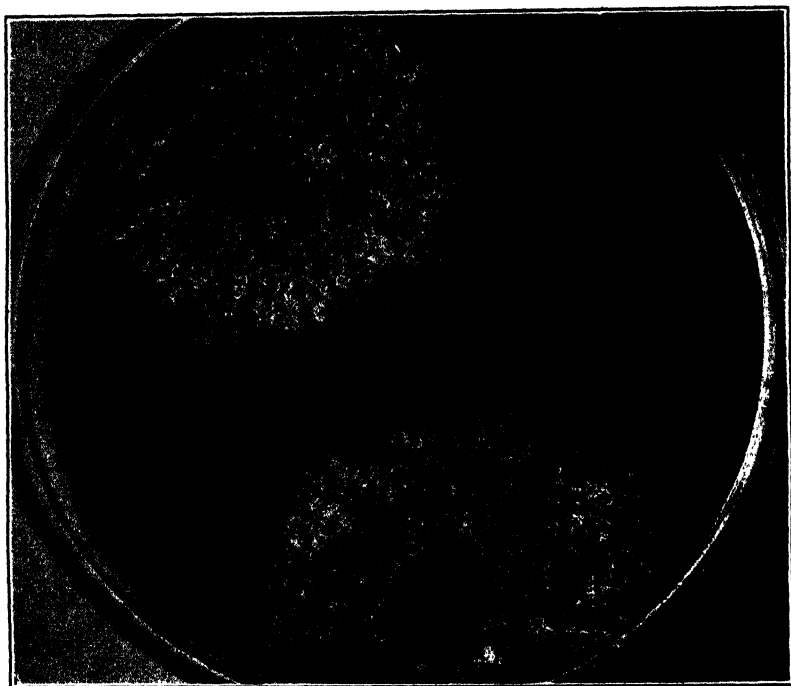


FIGURE 1.—*Trichosporium symbioticum* on malt agar, showing white flocculent air mycelium and dark submerged hyphae. Natural size.

MORPHOLOGY

In malt agar (pH 5.5) the dark-brown submerged mycelium is plainly septate and in old cultures the hyphae may obtain a maximum width of 14μ , with an average of 6μ . The whitish aerial hyphae are more delicate, ranging from 1.5μ to 2μ in width, and are also septate. Conidia are distinct from the conidiophores and occur in capitate clusters at their tips but occasionally subterminally as well. The spores are attached by the tips and are permanently hyaline, subglobose to ovoid in shape, and 1.5μ to 4μ in diameter. The conidiophores often divide into two or more branches, each of which may bear a capitate group of spores on the apices as illustrated in figure 2.

Microtome sections of infected wood, stained by the Cartwright (6) and Hubert (10) methods, when examined microscopically showed the presence of *Trichosporium symbioticum* hyphae within the tracheids,

and occasionally conidiophore and spore formations were also detected. Figure 3 is a photomicrograph showing *T. symbioticum* hyphae and spores within the tracheids of *Abies concolor* wood. The hyphae effect an entrance into the tracheids through the bordered pits and not directly through the cell walls. Hyphae also enter the medullary ray cells through the pits but are not as typically congregated here as is frequently the case with other wood-staining organisms such as *Ceratostomella* spp. Hyphae have been seen in the phloem parenchyma, but only indistinctly.

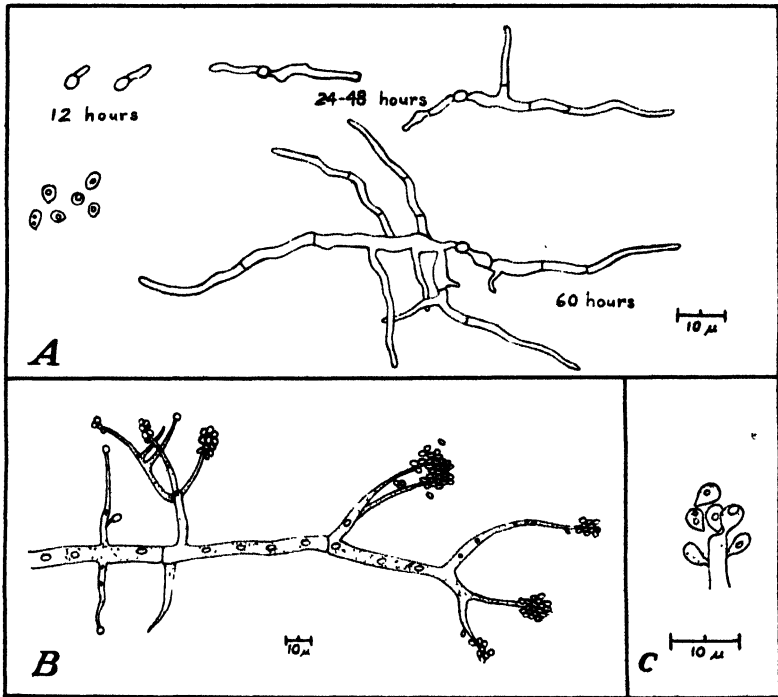


FIGURE 2. — *Trichosporium symbioticum* on malt agar. A, Spore germination and mycelial growth at 22° C.; B, typical aerial mycelium and conidiophores; C, enlarged tip of a conidiophore, showing manner of spore attachment.

In the tracheids of *Abies concolor* wood the maximum width of mycelium observed is approximately 8μ, averaging about 3μ. The spores produced in the tracheids are subglobose and vary from 1.8μ to 2.4μ in diameter. The sizes of the spores and hyphae do not differ as much within the tracheids as when the organism is grown in culture media.

OCCURRENCE OF STAIN IN GALLERIES OF *SCOLYTUS VENTRALIS*

To determine the frequency of stain occurrence in relation to *Scolytus ventralis* galleries, five infested trees were examined after they had been felled and the bark had been removed. These trees ranged from 8 to 30 inches in diameter. In all, 532 galleries were inspected macroscopically in consecutive order, and the brown discoloration was plainly evident in every case.

Special attention was given to evidences of overcome and unsuccessful beetle attacks, generally characterized by isolated galleries, to determine whether the brown discoloration was noticeable, thereby indicating the presence of *Trichosporium symbioticum*. In no instance was the coloration found to be entirely lacking, although the areas stained in unsuccessful attacks appeared to be less extensive than in successful infestations.

In trees where the beetle attacks are but few or have been unsuccessful, the stain is soon overgrown by newly formed xylem so that it

becomes buried under successive layers of annual growth. Years later, when such trees are felled and bucked, the stained areas may become evident on the cross cuts. The date of the attacks may be readily established by counting the number of rings that have been formed over the stain (fig. 4).

The trap trees⁶ used by the entomologists to attract *Scolytus ventralis* provided another means of studying the frequency of the occurrence of the fungus with individual egg galleries. Several weeks after the attacks were made the bark was removed from seven different trap trees and 999 galleries were examined successively. Stain was

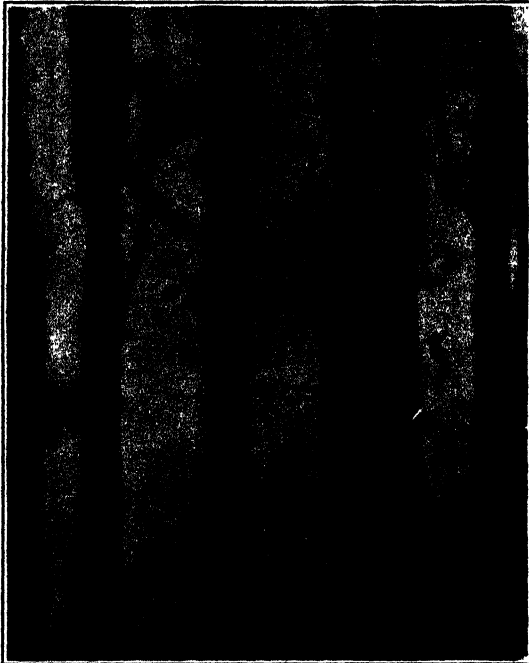


FIGURE 3.—Hyphae and spores of *Trichosporium symbioticum* within tracheids of *Abies concolor* wood. $\times 450$

plainly evident in all but 28, and these had been formed very recently. The number of trap-tree galleries inspected plus the number of galleries found in the infested firs makes a total of over 1,500 attacks examined. The stain was unquestionably present in all but 2 percent of the galleries, and the recent origin of these may explain its visual absence, since cultural isolations have already shown that the fungus was probably present in these doubtful cases.

At the time of the examination of the trap-tree attacks, measurements were made of the extent of the stain. Estimates of the approximate age of the egg galleries were based on their length. It was found that the discoloration spread longitudinally at an average rate of approximately 3 mm a day. It was possible to follow the rate at

⁶ In this investigation a trap tree consisted of a purposely felled, living white fir that was left unlopped on the ground. Unlopped trees are especially attractive to *Scolytus ventralis*, and by using this method forest entomologists secured abundant infestations at will.

which the stain spread for about 1 month, but after that time the discoloration of adjacent galleries ran together (fig. 5). The measurements indicated that individual trap trees show considerable variation in the rate at which the stain spread from the galleries.

The beetles extend their mines as the eggs are laid, and the stain is therefore first noticeable in the older portions of the galleries. Before any of the eggs hatch into larvae, however, the brown discoloration spreads longitudinally from 30 to 50 mm on both sides of the egg gallery. With this start, the stain keeps well in advance of the fastest working larvae. The writer has never found larval mines that have extended into unstained areas.

It appears from these examinations that when *Scolytus ventralis* attacks white fir a brown stain is regularly associated with the galleries of this beetle. Discoloration of the same sort has never been found except in connection with beetle infestations.

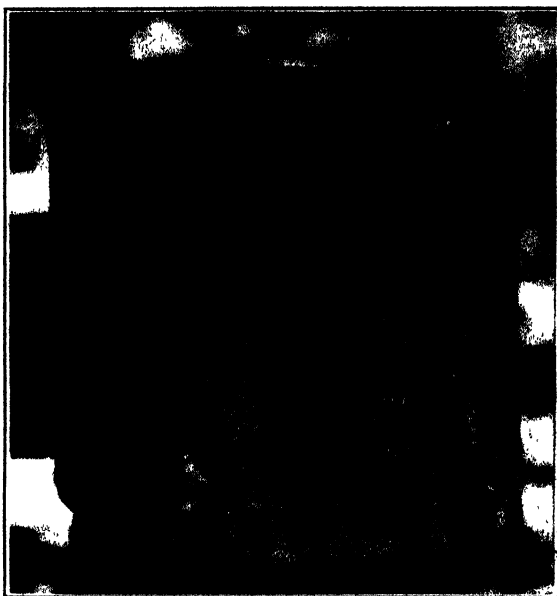


FIGURE 4.—Section of *Abies concolor* tree heavily mined with *Scolytus ventralis* galleries. Dates indicate year of attack. Notice callus formations surrounding the overcome and unsuccessful attacks. Extent of stain associated with the 1931 attacks was outlined by the writer. Photographed by George R. Struble; approximately one-fifth actual size.

THE BEETLES AS CARRIERS OF THE STAINING FUNGUS

ISOLATIONS FROM LIVING BEETLES

Infested logs placed in the Bureau of Entomology and Plant Quarantine rearing cages provided a ready supply of beetles. The adults emerged into small glass jars on the front of the cages, and 100 beetles were collected in this manner. Each insect was placed in a 50-mm Petri dish containing lukewarm malt agar, where they swam about until the agar solidified. The beetles were then removed with sterile forceps and the plates were incubated. Microscopic examinations of the plates were made periodically.

A number of different fungi were found growing in the plates, but 90 percent produced cultures of *Trichosporium symbioticum*. The most common contaminants were *Penicillium*, *Aspergillus*, and *Mucor* spp., none of which occurred consistently. In a few instances the plates contained practically pure cultures of *T. symbioticum*.

The beetles in the above investigation were not collected singly, and there was a possibility of mutual contamination. To obviate this objection 25 additional beetles were removed from the bark of infested

white fir before they emerged, and were placed in separate sterile test tubes preparatory to cultural proceedings. Later they were removed from the test tubes into lukewarm agar and allowed to swim about as previously described.

These beetles all proved to be carriers of *Trichosporium symbioticum*. It may be assumed, therefore, that *Scolytus ventralis* commonly carries the staining fungus and in this way introduces it into the egg galleries.

It appears that an occasional beetle that may emerge free of the fungus is likely to become a carrier afterward through contact with other beetles.

ISOLATIONS FROM ALIMENTARY TRACTS

To determine whether the beetles carried *Trichosporium symbioticum* internally or externally, 50 *Scolytus ventralis* alimentary tracts were cultured by means of the following technic: Before the beetles were dissected they were sterilized according to a method used by Beckwith and Rose (2), who studied the intestinal flora of termites. The living beetles were placed in a U. S. P. tincture of iodine solution for 20 to 30 seconds. They were then washed in two changes of physiological saline solution and finally in two changes of sterile water. The beetles were dissected under sterile conditions by Struble. Each alimentary tract was plated immediately on

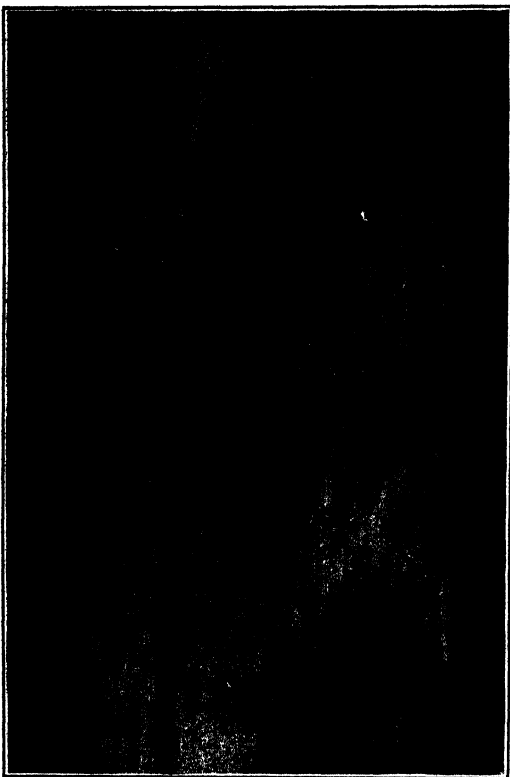


FIGURE 5.—Stain in the wood of an *Abies concolor* tree attacked by *Scolytus ventralis*. The stain is shown well in advance of the longest larval galleries, and in several cases the discoloration has spread from one colony to another, forming extensive areas. Photographed by J. M. Miller. Approximately one-fourth actual size.

malt agar by the writer. The plates were examined regularly during incubation.

Trichosporium symbioticum developed from only 4 of the 50 alimentary tracts. The small number of positive isolations indicates that the fungus is not commonly carried internally.

PATHOGENICITY TESTS

As a test of the pathogenicity of the isolated fungus, Struble⁷ drilled a number of white fir trunks with a $\frac{3}{32}$ -inch bit to simulate the beetle galleries, and inoculated through these openings. The writer inoculated seven trees in a similar manner, mainly to determine the

⁷ STRUBLE, G. R. See footnote 3.

rate at which the stain spread. The surface of the bark was sterilized with 70-percent alcohol and the drillings were made horizontally in an ascending spiral to avoid the effect of mechanical girdling. The inoculum, consisting of *Trichosporium symbioticum* in bits of malt agar, was inserted into the drill holes with a sterile needle. Check insertions of sterile agar were alternated with the inoculations in the same trees. Both the inoculations and checks were left undisturbed for 3 to 8 weeks. Then the bark was cut away to expose the stain and in some cases the trees were felled and the bark entirely stripped from the trunks. This revealed the presence of stain very clearly (fig. 6).

Measurements from a total of 145 inoculations showed that the stain spread only approximately one-third as rapidly as the discoloration associated with the beetle galleries, the daily spread averaging 1 mm.

All the checks except 5 in 1 tree remained free of discoloration. The trunk of this particular tree was enclosed with a wire screen after inoculation, and a number of *Scolytus ventralis* adults were introduced later into this cage by the entomologists, to determine whether the beetles preferred stained or unstained areas in which to establish their egg galleries. The experiment failed to settle the question, possibly because of the presence of excessive balsam,⁸ which had exuded from the drill wounds and which apparently restricted the beetles from making extensive explorations. Since both the checks and the inoculations were left open, it is possible, however, that exploring beetles did introduce the fungus into the five checks.

Reisolations were taken from four of the inoculated trees and reinoculated into other unstained white fir trees. Subsequent examinations revealed the presence of the brown discoloration in all reinoculations.

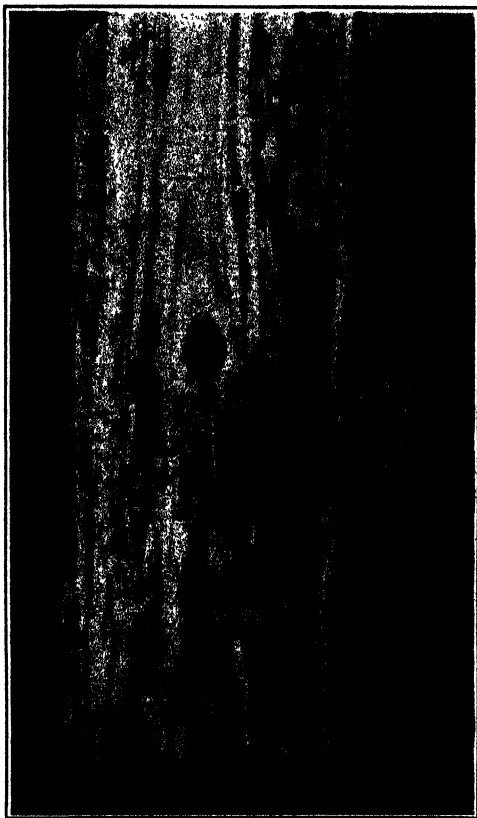


FIGURE 6.—Section of *Abies concolor* trunk, inoculated by the drill method, from which the bark has been removed. On the left the checks show the absence of stain (indicated by check marks). On the right are two inoculations showing the spread of the stain in the sapwood (after 4 weeks). Approximately one-half actual size.

⁸ Preliminary tests by members of the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, have since shown that white fir balsam is actually very toxic to *S. ventralis* beetles.

Several other organisms, obtained during isolation studies, were also inoculated into white fir trunks but gave negative results.

CORK-BORER INOCULATIONS

To determine whether *Trichosporium symbioticum* could kill white fir trees without the aid of the beetle galleries, it was necessary to devise a method of inoculation in which the inoculum could be placed in the cambial region in such a manner that the stain would naturally spread to form a solid band around the tree trunk. To meet this requirement and to reduce the effect of mechanical wounding to a minimum, a cork-borer method of inoculation was employed (15).

Twenty white firs growing on the same site were selected, 10 to be inoculated and 10 to be used as checks. Considerable care was exercised in these selections to obtain check trees that were as nearly as possible duplicates of the trees inoculated. Before the inoculations were made, the portion of the stem to be inoculated was carefully washed with 70-percent alcohol.

The method consisted of using a no. 3 cork borer to cut out a series of bark disks extending entirely around the tree trunk. From one-half to 1 inch of bark was left between borings, to obviate mechanical girdling. The borer was forced through the bark down to the xylem and carefully withdrawn containing the bark disk in the barrel. The inoculum, consisting of *Trichosporium symbioticum* in malt agar, was deposited in the hole with a sterile needle, and the disk was immediately replaced by forcing it from the barrel directly into place with a plunger.

After the completion of a ring of borings, a strip of waterproof adhesive tape was placed over the disks This held each disk in position and prevented as far as possible the detrimental effect of drying out.

Since the fungus, as previously indicated by the stain spread, has only a limited lateral growth, it was necessary to arrange the inoculations in more than one ring. These were made about 2 to 3 inches apart, with the borings of the lower ring alternating with those of the



FIGURE 7.—*Abies concolor* tree 10 months after inoculation with *Trichosporium symbioticum* by the cork borer method. Balsam has started to flow from the base of the girdle, which was produced by the coalescence of the stain. This is tree 7 in table 2. One-third actual size.

ring directly above. In this way the uninoculated areas between the borings would be more certainly invaded by the fungus hyphae as they extended longitudinally.

The checks were treated in the same manner as the trees inoculated, except that sterile malt agar was used instead of the inoculum.

The results secured from the cork-borer inoculations are presented in table 2, along with other essential data. The condition of the trees was judged largely on the general appearance of the crowns. Final examinations were made 16 months after the beginning of the tests. Examinations of the inoculated trees were made by peeling off the bark from the inoculated portions.

Eight months after the beginning of the tests the bark adjacent to the inoculated areas was much cracked and there was a considerable flow of balsam. On several of the trees definite cankers had formed and callus formation had started. All of the check trees at the time appeared normal, and only a very slight superficial cracking of the bark could be detected, with no external balsam flow. By midsummer of the year following the initiation of these tests, some of the inoculated trees had failed to produce new needles and a few dead branches could be seen in the crowns. The infected trees all showed pronounced callus formations accompanied by definite cankers and heavy flow of balsam from the region of inoculation.

TABLE 2. -Results of cork-borer inoculations after 16 months

Tree no.	Diameter breast high	Vigor	Borings	Bands	Condition ¹
	Inches		Number	Number	
1.....	4½	Medium.....	13	2	Dead.
2.....	3½	do.....	11	2	Normal.
3.....	5	do.....	13	3	Several dead branches.
4.....	2½	do.....	6	2	Dying.
5.....	2	Poor.....	5	2	Normal.
6.....	3½	Medium.....	11	1	Do.
7.....	3	Poor.....	14	2	Dead.
8.....	3½	Medium.....	11	2	Normal.
9.....	4	do.....	12	2	Do.
10.....	4½	do.....	16	2	Dead

¹ Condition of all checks was normal.

Detailed examinations of all the inoculated trees showed that the cambium was killed as the fungus advanced and that when the stain had coalesced into a solid band extending completely around the tree trunks an effective girdle was formed. It was also found that when the inoculated trees did not show indicative dead branches in the crown the stain had failed to coalesce in one or more places on the trunks; hence the girdles were not complete, and the trees survived. It should be mentioned, however, that before any of the inoculated trees succumbed *Scolytus* beetles had attacked the trunks above the girdles and probably hastened the death of the trees. Figure 7 shows the appearance of an inoculated tree trunk at the conclusion of the test. The checks remained in normal condition throughout the entire period.

From these tests it may be concluded that *Trichosporium symbioticum* is definitely pathogenic. Mechanical wounds, such as those pro-

vided by the beetle galleries, may aid the fungus in becoming established but are not essential for the extension of the hyphae. The fungus, spreading ahead of the galleries in beetle-infested trees, kills the cambium as it advances. When the stain coalesces to form large areas, spreading from one gallery to another, it produces a more effective girdle than would the galleries alone.

Others have also shown that wood-staining organisms may be parasitic on the host. Münch (11) concluded that under certain conditions the bluing fungi, with which he worked, may cause the death of pine trees. Nelson and Beal (12), working with southern pine in this country, showed that the blue stains associated with *Dendroctonus frontalis* Hopk. may play an important part in killing infested trees. The organisms studied by the above workers did not belong in the genus *Trichosporium*, but other investigators have indicated certain of these species to be parasitic as well. Butler (5) found *T. vesiculosum* Butl. associated with the death of casuarina trees in India. He found that the hyphae lie within the vessels and bore their way through intervening cell walls. In other instances, species of *Trichosporium* have been indicated to be parasitic, such as *T. parasiticum* Dearn. and Bisby (4) on *Amelanchier alnifolia* Nutt. leaves.

Since stain development precedes the appearance of the larvae, it may be that the fungus aids the brood by adjusting localized conditions. This seems to be particularly pertinent in isolated galleries, such as overcome attacks, where the host tree remains alive but the larvae thrive.

The killing of the cambial region probably restricts the flow of balsam, or the hyphae of *Trichosporium symbioticum* may render certain food products more digestible, as shown by Heitz (8) and others for other fungi. The effect of the stain on moisture conditions may also be of particular importance, as suggested by Struble,⁹ since the larvae have never been found in unstained material. It appears desirable, therefore, to determine what effect the fungus may have on moisture conditions within infected areas.

MOISTURE RELATIONSHIP TESTS

The cork-borer method was employed again to construct a partial band of *Trichosporium symbioticum* inoculations extending around only one-quarter of the circumference of several trees. Two rings were spaced 2 inches apart and the borings of the lower ring alternated with those above, as described before. Ten white firs were inoculated in this manner, and the disks were sealed in place with adhesive tape.

These inoculations were made in late June and were not disturbed for 2 weeks since a similar length of time elapses between the initiation of a *Scolytus ventralis* attack and the appearance of the first larvae. At that time the adhesive tape was removed and a 1-inch arch punch¹⁰ was driven through the bark between the inoculation rings and into the sapwood where the stain would be coalesced. In this manner a core of stained wood and overlying bark was readily removed from five inoculated trees and to prevent desiccation was

⁹ See footnote 3.

¹⁰ An arch punch is a steel tool used in the leather trade. It has a sharp, hollow, circular blade over which the handle arches; hence the name.

wrapped in tin foil before being deposited in a container which was then tightly closed. Similar cores of fresh unstained wood were extracted about an inch from the end of the inoculated areas. Since the discoloration does not penetrate deeper than the current growth ring, the specimens were trimmed down to the thickness of one ring and the bark was removed before weighing. The cores were dried at 100° C. until a constant weight was obtained for each sample, and the percentage of moisture was determined on a dry-weight basis. Table 3 summarizes the comparative results of the final weighings.

TABLE 3.—Comparison of moisture in stained and unstained white fir wood 2 weeks after inoculation

Tree no	Diameter breast high	Unstained wood			Stained wood			Difference in moisture
		Wet weight	Dry weight	Moisture ¹	Wet weight	Dry weight	Moisture ¹	
	Inches	Grams	Grams	Percent	Grams	Grams	Percent	Percent
11	9	1 321	0 434	204	1 128	0 569	98	-106
12	7	1 388	.494	187	618	.320	93	-94
13	6	1 474	.482	206	1 550	.672	131	-75
14	8	1 753	.509	193	1 788	.760	135	-58
15	7	2 134	.684	212	2 050	.920	123	-89
Average	7.4	1 614	.536	200	1 427	.648	116	-84

¹ Percentage of moisture based on dry weight.

After 2 months it was found that the moisture in stained samples from five other white firs still averaged 88 percent less than that of unstained wood taken from the same trees.

It is known that moisture fluctuates considerably in individual trees, depending upon the time of the day and the season, and that there may also be variations even in the same tree trunk, particularly with reference to the circumference. These percentages are, therefore, presented as approximate rather than absolute. The fact remains, however, that *Trichosporium symbioticum* does reduce the moisture conditions locally and that the beetle larvae develop where moisture is less than in adjacent unstained areas, since their galleries occur only in association with the discoloration. It is not impossible that the fungus may consequently render conditions more favorable for the successful development of the larvae and particularly aid in the preservation of isolated colonies.

CONCLUSIONS

On the basis of the data collected, the writer suggests that the relationship of *Trichosporium symbioticum*, n. sp., to *Scolytus ventralis* Lec. may be one of mutual advantage.

The beetle benefits, since the fungus kills the cambium as it advances and thereby assists in overcoming the host tree. The fungus reduces the moisture content of the cambial region ahead of the larvae and in this way may render conditions more favorable for their development.

The fungus benefits, since it is carried by the beetles to new and suitable substrata, presumably otherwise unattainable, where it is able to maintain and reproduce itself. The association appears to be symbiotic.

SUMMARY

A fungus has been frequently isolated from a number of brown-stained areas associated with *Scolytus ventralis* Lec. galleries. The isolated organism has been determined as *Trichosporium symbioticum*, n. sp.

The stain is regularly associated with *S. ventralis* galleries in white fir and has always been observed to precede the appearance of the larvae.

S. ventralis adults carry *T. symbioticum*, mostly epizootically, and in this way presumably spread the fungus from infected to noninfected trees.

A cork-borer method of inoculation has shown that *T. symbioticum* is definitely pathogenic and capable of killing the cambium as it advances. In this way it may aid the beetles in overcoming the host trees. The hyphae, spreading ahead of the larvae, reduce the moisture content of the areas in which the larvae extend their mines.

The regular association of *T. symbioticum* with *S. ventralis* appears to be one of mutual benefit and suggests a symbiotic relationship.

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A COMPARATIVE STUDY OF CERTAIN MORPHOLOGICAL CHARACTERS OF SUGARCANE \times SORGO HYBRIDS ¹

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INTRODUCTION

Breeding work was initiated by the writer (3, *Rept.* 45)² in the fall of 1930 for the special purpose of obtaining crosses between sugarcane, *Saccharum officinarum* L., and sorgo (sweet sorghum), *Holcus sorghum* L. var. *saccharatus* (L.) Bailey. Earlier in the same year Thomas and Venkatraman (14) announced that they had succeeded in securing crosses between sugarcane and *Sorghum durra* Stapf. The first attempt to obtain crosses in Florida proved unsuccessful and led to a detailed study of the mechanics of cross-pollination under local conditions, the main results of which will be reported briefly. Since sugarcane blooms in this hemisphere only when daily illumination is short, starting usually in late November or early December and lasting until late February, this intergeneric cross can only be effected each year during this particular period in the annual cycle.

During the flowering season of 1931-32, the cross was again attempted. Certain information gained in the previous trials was used, and approximately 145 hybrids were secured, only 69 of which were actually set in the field for growth to maturity, as previously reported by the writer (3, *Rept.* 46). Six of those set in the field failed to reach maturity, so that only 63 were available for study, 24 being of the cross P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo, and 39 of the cross P. O. J. 2725 sugarcane \times Early (Kansas) Orange sorgo.

During the flowering season of 1932-33 the crossing work was repeated, and approximately 200 hybrids were secured. Of these, 84 of the cross P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo and only 6 of P. O. J. 2725 sugarcane \times Sugar Drip Sorgo reached the potted stage and were planted in the field. Further deaths in the field reduced the total number of hybrids reaching maturity in this series to 65.

It is therefore seen that the total number of hybrids grown to maturity was 128 from a total population germinated in the seed bed of 345, or only 37 percent. The majority of the young plants, including a number of albino types, died shortly after germination, apparently due to genetic weakness or incompatibility.

During the flowering season of 1931-32, the reverse cross—Texas Seeded Ribbon sorgo (female) \times C. P. 27-35³ (male) sugarcane was attempted. In this trial, 97 sorgo flowers were carefully emasculated just before the spikelets reached maturity, and dusting with sugar-

¹ Received for publication July 19, 1934; issued May 1935.

² Reference is made by number (italic) to Literature Cited, p. 551.

³ A male parent having an abundance of healthy pollen. This sugarcane is a cross between D. 74 (female) \times U. S. 1664 (male), produced by the writer at the U. S. Sugar Plant Field Station at Canal Point, Fla., in 1927.

cane pollen was carried out in an isolated area free from sorgo pollen. This single reciprocal crossing trial, however, resulted in failure.

MATERIALS AND METHODS

In the main, the hybrid material used for the studies here reported consisted of crosses between P. O. J. 2725 sugarcane (female) × Texas Seeded Ribbon sorgo (male). The plants were grown on Okeechobee muck soil adjacent to plots of the parental types at Lake Harbor, Fla. The age of the hybrids and of P. O. J. 2725 sugarcane grown for comparison was approximately 1 year from the germination of seeds and cuttings, respectively. The sorgo plants grown for comparison, however, were almost 4 months old from seed planted in the fall and grown during the cool, dry winter months when the daily illumination is short. Comparisons have not been made of hybrids with sorgo plants grown during the warm, moist spring and summer months when the daily illumination is long.

The sugarcane variety P. O. J. 2725, used as a female parent in the hybridization experiments was previously found to be almost completely male sterile in repeated trials. Occasionally 1 or 2 seedlings have been produced per tassel under conditions where cross-pollination was impossible.

Physiological studies on the germination of sugarcane and sorgo pollen were conducted similar to those on sugarcane pollen by Dutt and Ganapathi Ayyar (6) and Weller (16). It was found that the best artificial medium on which to germinate sorgo pollen at 23° C—the temperature at which all comparative trials were made—consisted of 34 g of sucrose, 66 g of distilled water, and 0.3 g of agar. The medium when made up had a reaction of pH 5.6. Weller (16) found that the best artificial medium for the germination of sugarcane pollen in Hawaii consisted of sucrose 25.0 g, water 100.0 cc, and agar 0.35 g. This medium, which contains 19.5 percent sucrose, was found to germinate the pollen of several varieties of sugarcane grown locally. Since the medium used by Dutt and Ganapathi Ayyar (6) for germinating sugarcane pollen contained 26 percent sucrose and 0.7 percent agar, it would appear that the optimum medium for the germination of sugarcane pollen varies according to the climatic conditions under which the plants are grown. The data also suggest that even different sugarcane varieties may have different medium preferences under uniform external environmental conditions. A comparison of the results with the germination of sugarcane pollen from India and Hawaii would also tend to explain the success with the intergeneric cross between sugarcane and sorgo in the former country, since it is evident that sugarcane stigmas in India have an osmotic pressure very much nearer the optimum for sorgo pollen germination than in either Hawaii or this part of Florida.

Basing calculations on the researches of Frazer and Myrick (7) on the osmotic pressure of sucrose solutions, it was found that the medium suitable for the germination of local sugarcane pollen at 23° C. has an approximate osmotic pressure of 18.5 atmospheres and that for sorgo pollen, 41.5 atmospheres. The experimental evidence indicates clearly, therefore, that for success in effecting cross-pollination of sugarcane and sorgo, the medium on the sugarcane stigmas should have its osmotic pressure more than doubled in order to provide an

ideal physical environment for the sorgo pollen to germinate under local conditions.

In all the germination studies with local sorgo pollen it was found that while good germination was secured with cells shed between 6 and 8 a. m., the percentage of germination fell off rapidly after 8 a. m. Although no actual germination trials were reported by McIntosh (12), this author showed that the optimum period for pollen shedding of sugarcane in Barbados is between 7 and 8:30 a. m. This period agrees fairly well with that at which sorgo pollen germinates best locally.

For effecting cross-pollination between sugarcane and sorgo, the sugarcane tassels were bagged from late evening until about 10 a. m. each day throughout the flowering period, so as to exclude rain and

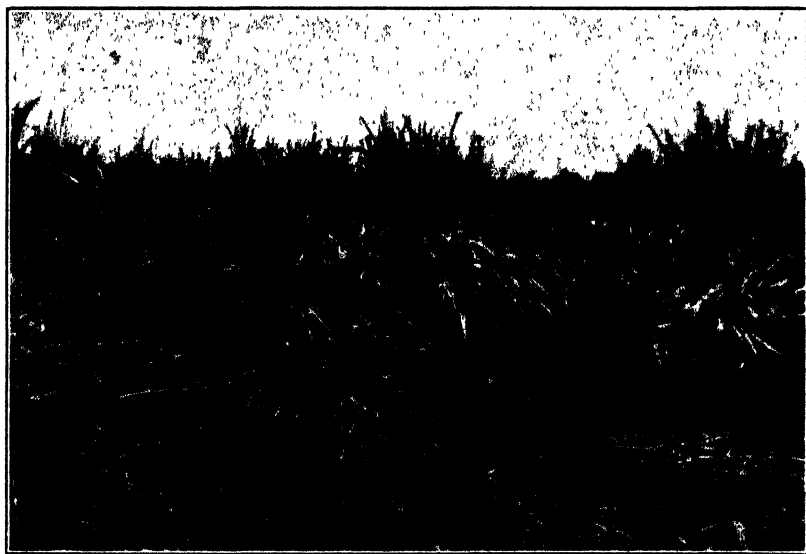


FIGURE 1. --General view of a portion of seedling field showing hybrids of P. O. J. 2725 sugarcane × *Holcus sorghum* var. *saccharatus*. Age of plants about 1 year from the germination of seed. In the back ground at the left is a sugarcane plot which has arrowed.

dew and prevent reduction in osmotic pressure of the stigma fluid. The sorgo pollen was dusted on the tassels under the bags between 6 and 8 a. m. After the pollination period of about 10 days was over, the sugarcane tassels were allowed to ripen normally in the open, this operation usually requiring approximately 4 weeks.

For germinating the mature seeds, only steam-sterilized muck soil and flats were employed, and the most favorable conditions of moisture, temperature, and greenhouse protection were provided.

The method of selecting, preparing, and staining epidermal material for anatomical characters was essentially the same as that used by Artschwager (1). The staining of the epidermis of the stem of sorgo with chloriodide of zinc had to be performed under cover glasses because of the marked tendency of films to curl rapidly.

In order to obtain an estimate of the variance occurring among hybrid populations and parent plants in those factors studied, a determination was made of the standard error of the means with a

view to providing evidence of significance where this occurred. The usual formula, $\frac{\sigma}{\sqrt{n}}$, was used in all the calculations reported.

COMPARISON OF GENERAL CHARACTERS OF P. O. J. 2725 SUGARCANE \times TEXAS SEEDED RIBBON SORGO HYBRIDS WITH THEIR PARENTS

Figure 1 gives a general view of a portion of a seedling plot of sugarcane \times sorgo hybrids (P. O. J. 2725 sugarcane \times *Holcus sorghum*



FIGURE 2.—One of the best selections of the cross P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo in bloom. This is selection F. 32-47.

var. *saccharatus*) about a year after the germination of the seed. In general appearance these resemble the hybrids between P. O. J. 2725 sugarcane \times *Sorghum durra* produced by Venkatraman and Thomas (15). As may be observed, a majority of them are dwarfed types which do not appear promising for commercial utilization.

Only about 3 percent showed enough vigor and field characters of sufficient promise to be considered for further trials. One of the types, selection F. 32-47, selected for propagation and testing, is shown in figure 2. About 1 percent of the population consisted of types which could not be distinguished from true sugarcane seedlings. These latter might have been produced either parthenogenetically or through normal selfing.

WIDTH OF LEAVES

Venkatraman and Thomas (15) have already noted certain characters, such as shortness, softness, and light green color of leaves of hybrids between P. O. J. 2725 sugarcane and *Sorghum durra*. Considerable variation was noted in regard to these same characters among the progeny of P. O. J. 2725 sugarcane × *Holcus sorghum* var. *saccharatus*, representing almost a complete series from one parent to the other. Leaf width was studied in a population of 59 crosses between P. O. J. 2725 sugarcane and Texas Seeded Ribbon sorgo. The number of progenies having leaf widths falling in eight groups is recorded in table 1. The modal class of progenies occurred in the 4.5-cm group. The average of all progenies was 4.95 ± 0.17 cm, while the average leaf width of P. O. J. 2725 sugarcane and Texas Seeded Ribbon sorgo planted on the same soil type and at the same time was 8.2 ± 0.31 cm and 2.8 ± 0.07 cm respectively. Thus the average width of the leaves of the hybrid progeny is not significantly greater or less than the average of the two parents.

TABLE 1.—Grouping of hybrids between P. O. J. 2725 sugarcane and Texas Seeded Ribbon sorgo according to leaf width¹ (59 hybrids), height² (58 hybrids), and stem width (49 hybrids)

LEAF WIDTH								
Item	Number falling in class (cm) indicated							
	1.75	2.5	3.5	4.5	5.5	6.5	7.5	8.5
Progeny	1	3	8	22	14	7	3	1

HEIGHT														
	10	30	50	70	90	110	130	150	170	190	210	230	250	270
Progeny	2	18	6	13	7	3	4	1	2	0	0	1	0	1

STEM WIDTH					
	1.25	1.75	2.25	2.75	3.25
Progeny	6	18	10	12	3

¹ Measurements made in the middle of blade at point giving the maximum width; approximately 10 leaves of each hybrid were measured.

² Average of all primary shoots measured from base to sheath-blade joint of the first expanded apical leaf.

HEIGHT OF PLANTS

It has already been noted that the majority of the sugarcane × sorgo hybrids that grow are rather stunted and not commercially promising in appearance. In order to gain a better idea of the varia-

bility within a population of a given cross, the height of 58 progenies of P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo was studied when the plants had matured, which was about 1 year after the germination of the seed. The height of all the primary shoots was found by measuring from the base to the sheath-blade joint of the first expanded apical leaf. The number of plants falling in 14 groups and the modal class are recorded in table 1.

The results show that the modal class of progenies, or 31 percent, fell in the group of 30 cm height. The next largest percentage (22 percent) fell in the group of 70 cm. Variations from 15–280 cm were recorded, the average of all 58 progenies being 75.7 ± 6.7 cm.

Texas Seeded Ribbon sorgo grown at the same time and on the same soil type (growth period from October to February) averaged 150.0 ± 5.3 cm in height when in bloom. Normal P. O. J. 2725 sugarcane, as first crop or plant cane of the same age as the hybrids and growing on the same soil type in an adjoining plot, averaged 316.0 ± 13.8 cm in height. Thus the average height of the two parents was nearly three times that of the average of the 58 progenies resulting from the crossing of these parents. Had the sorgo for this comparison been grown in the spring and summer, instead of fall and winter, when opposite conditions of moisture, temperature, and duration of daily illumination occur, very different results, and results of even greater significance, might probably have been obtained.

DIAMETER AND OTHER CHARACTERS OF STEMS

There is almost a complete series of variations in stem characters among the hybrids, ranging from those resembling sugarcane to those resembling sorgo. Stalk diameter, shape of bud, width of root band, and depth and extent of bud grooves on the stem internodes vary to a considerable extent. Typical variations in all of these characters are shown in figure 3. The bud groove on the internode, a very prominent character of the sorgo parent only, appeared to be one of the most dominant characters. Among 47 progenies of P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo, 41 exhibited marked grooves, while only 4 showed slight grooves and 2 showed none.

A study was made of 49 progenies of P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo with regard to diameter of stalk midway between the apex and base. The number of progenies falling in each of five groups is recorded in table 1. The modal class occurred in the 1.75-cm group. The average of all 49 progenies was 2.23 ± 0.08 cm, while that of P. O. J. 2725 sugarcane was 3.23 ± 0.05 cm and that of Texas Seeded Ribbon sorgo was 1.26 ± 0.07 cm. The average for the two parents was 2.24 ± 0.12 cm. Thus, the stalk diameter of the progeny of this cross was, on an average, intermediate between that of the two parents, with some extremes occurring in the groups nearest sugarcane and some in the groups nearest sorgo.

PECULIARITIES OF INFLORESCENCES

As in the case of sugarcane, hybrids which "boented" ⁴ or bloomed did so only during that period in the annual cycle in which daily illumination was short. Relatively few hybrids have so far produced

⁴ This is a term frequently used in sugarcane literature when referring to the tapered or spiked appearance of the apical growing point of the stalk prior to the emergence of the inflorescence.



FIGURE 3.—Middle stalk portions of four sugarcane \times sorgo hybrids: A, F. 31-61 (P. O. J. 2725 \times Early Orange); B, F. 31-6; C, F. 31-10; D, F. 31-13 (P. O. J. 2725 \times Texas Seeded Ribbon). The first two have very shallow furrows which extend about halfway between nodes as in the case of the sugarcane parent P. O. J. 2725. The last two have narrow but very deep furrows, which frequently extend from node to node as in the case of the sorgo parent, Texas Seeded Ribbon sorgo.

inflorescences, although many have boented during the past two tasseling seasons. Typical blooms of four different hybrids are shown in figure 4. Among these, the strain F. 31-13, resulting from the cross P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo (figure 4, C), grown in 1932, showed many of the interesting characters reported by Venkatraman and Thomas (15) for certain seedlings of the cross P. O. J. 2725 sugarcane \times *Sorghum durra*. These authors mention that 22 percent of their hybrids that arrowed showed an awned fourth glume as well as such abnormalities as aborted or multiple ovaries and pistils with a single stigma or with four stigmas. So far as the writer is aware, this is the first record of a four-stigma

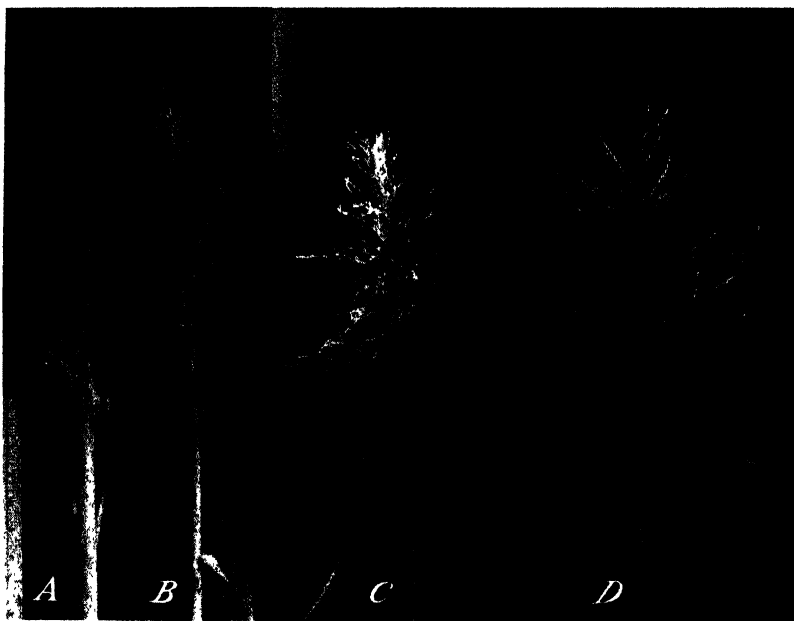


FIGURE 4 --Blooms of four sugarcane \times sorgo hybrids. A, Tassel of F. 31-30 (P. O. J. 2725 sugarcane \times Early Orange sorgo), B, tassel of F. 31-10 (P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo); C, tassel of F. 31-13; D, tassel of F. 31-29, both C and D being P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo.

floret or of a multiple ovary among those members of the Gramineae which possess uniflorous spikelets. Hitchcock, in Gray's New Manual of Botany (8), records only the rare occurrence of 1 to 3 styles to the pistil among the Gramineae and apparently has never observed any variation whatever from the single pistil with its one-celled, one-ovuled ovary in each floret.

The occurrence of multiple ovaries is to be distinguished from true polyembryony, an instance of which has recently been noted by Dutt (5) in a cross between two sugarcanes.

Stratton (13) has shown that while each spikelet in *Zea mays* normally bears two flowers, one functional and the other aborted, in *Z. mays* var. *polysperma*, both flowers may function to produce either a pair of separate connate seeds (kernels back to back) or semiconnate seeds. A condition of abnormal spikelets similar to that in *Z. mays*

var. *polysperma* has also been noted by Karper (11) in the case of *Andropogon sorghum*, good evidence being furnished that the extra kernels arose from additional functional flowers within the spikelet. It should be noted especially, however, that Karper recorded spikelets with twin seeds, coalesced twin seeds, and triplet seeds among several varieties of grain sorghum, but did not find any multiple-seeded spikelets among sorgho varieties. The presence of multiple seeded spikelets in both *Z. mays* var. *polysperma* and *A. sorghum* should not be confused with the situation occurring in the hybrid F. 31-13, however, for in the latter case, each spikelet is uniflorous, exactly as in sugarcane, and does not possess additional aborted flowers, so that a multiple ovary, or a biovaried condition, as well as the occurrence of three or more styles or stigmas to the floret must be regarded as being especially significant.

STRUCTURE OF SPIKELETS OF PARENTS

In P. O. J. 2725 sugarcane, two uniflorous spikelets are alternately placed at the nodes of the rachis, one being sessile and the other stalked. Both spikelets are oblong and lanceolate and possess tufts of long, silky hair at their base. A cross section of a spikelet of P. O. J. 2725 sugarcane, illustrated in plate 1, *B*, also shows that this variety is normal and identical in structure with many varieties of *Saccharum officinarum*. There are present an outer and an inner glume, a sterile lemma (third glume), fertile palea, 2 thick lodicules, 3 stamens, and a single ovary. The fertile lemma (fourth glume), mentioned by Artschwager et al. (1) as being present in the hybrids of *S. spontaneum*, is absent, in spite of the fact that this variety has approximately one-eighth *S. spontaneum* blood (10). In the Andropogoneae, the tribe in which sugarcane is placed, Jeswiet (9) states that the fourth glume (g_4) is very rarely missing and is also generally present in the genus *Saccharum*, although in a great many varieties of *S. officinarum* it is absent.

The structure of the spikelet in Texas Seeded Ribbon sorgho has been described by Cowgill (4). The fertile spikelet in this variety has outer and inner glumes, sterile lemma, fertile lemma with awn medium in length and somewhat loosely twisted, 2 lodicules, a fertile palea, 3 stamens, and a single ovary. As compared with sugarcane spikelets, those of all sorgho varieties have a much greater diameter.

STRUCTURE OF SPIKELETS OF HYBRID F. 31-13

Like sugarcane, the hybrid F. 31-13 (P. O. J. 2725 sugarcane × Texas Seeded Ribbon sorgho) has two uniflorous spikelets alternately placed at the nodes of the rachis, one sessile and the other stalked. The spikelets are oblong and lanceolate and possess tufts of long, silky hairs at the base. As recorded by Venkatraman and Thomas (15) for 22 percent of their sugarcane × sorghum hybrids that arrowed, F. 31-13 showed an awned fourth glume or fertile lemma, as illustrated in plate 2, *A*. The awn is characteristically curved and is distinctly longer than the fertile lemma. While many flowers possess 2 purplish stigmas, very frequently 3 and 4 (pl. 3, *A*, *B*), and occasionally even 5, stigmas occur. Spikelets (uniflorous) with 4 or 5 stigmas possess two distinct ovaries, as illustrated in plate 3, *A*. Serial microtomic

sections show that when a biovaried condition occurs, one ovary may have its ovule wanting (pl. 2, *B*). In addition to the foregoing parts, this hybrid has outer and inner glumes, a sterile lemma, a fertile palea, 2 lodicules, and 3 anthers (stamens), as shown in plate 1, *A*. While in sugarcane the sterile lemma has been noted by Jeswiet (9) to be mostly without nerves or vascular bundles, and Artschwager et al. (2) state that the fertile lemma, if present, has no vascular elements, both the sterile and fertile lemmas of the hybrid F. 31-13 are nerved (pls. 1, *A*, and 2, *A*). The palea, however, is without vascular elements, which is in accord with the condition existing in the genus *Saccharum*. No flowers were observed to possess more than 3 anthers, even though they had more than 2 styles and stigmas as well as 2 ovaries.

Venkatraman and Thomas (15) mention that the glumes of P. O. J. 2725 sugarcane and its sugarcane hybrids as well as *S. spontaneum* are awnless, whereas *Sorghum durra* possesses a distinctly awned fourth glume. The same condition of an awned fertile lemma or fourth glume exists in *Holcus sorghum saccharatus*, variety Texas Seeded Ribbon, and it is therefore not surprising that the hybrid F. 31-13 exhibits this character. Jeswiet (9) records the frequent occurrence of a shorter or longer awn or needle among the Andropogoneae. He states that sometimes this needle is all there is and it may vary in length from a fraction of a millimeter to more than a decimeter.

Plate 1, *A*, *B*, shows the diameter of the spikelet of the hybrid F. 31-13 to be significantly greater than that of the sugarcane parent P. O. J. 2725, undoubtedly due to the influence of the large diameter of the spikelets in the sorgo parent, Texas Seeded Ribbon.

The vascular system of the rachis in sugarcane below the cushion joint has been shown by Artschwager et al. (2), in the case of 1 variety, to consist of 2 large bundles toward the center and 3 smaller ones toward the outside. In the hybrid F. 31-13, when 3 styles, 3 stigmas, and a single ovary are present, there are 5 smaller bundles in addition to the 2 larger bundles.

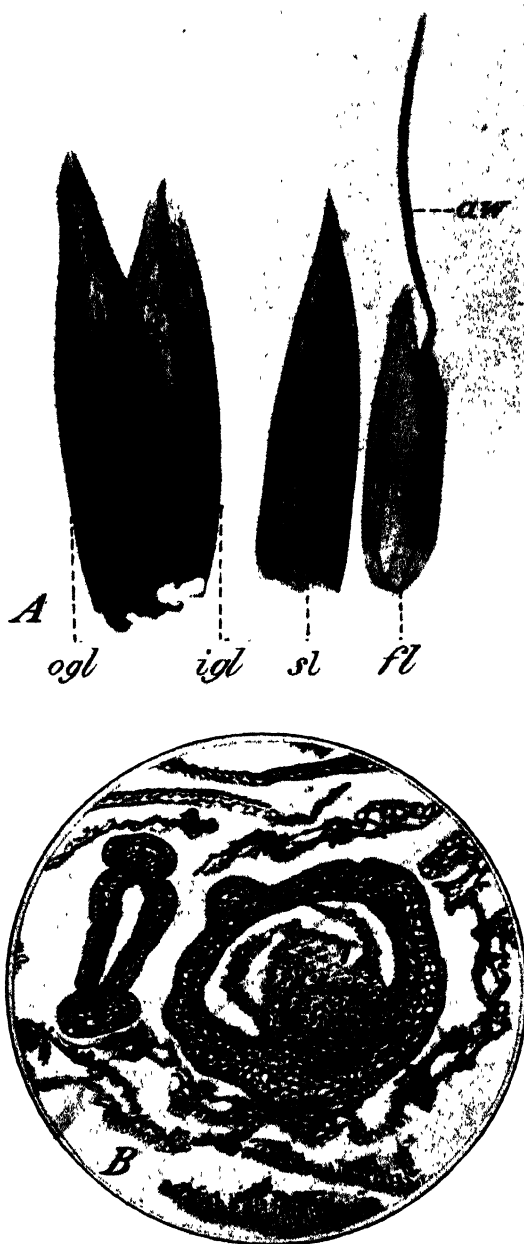
It has also been noted that the vascular system of the ovary wall of the hybrid F. 31-13 indicates clearly the number of styles and stigmas borne by the spikelet. In sugarcane, Artschwager et al. (2) note that the ovary wall has two fine lateral vascular strands running longitudinally through it. These, of course, are the strands which connect with the 2 styles and stigmas in the normal flower. In the hybrid F. 31-13, however, while the normal 2-stigma flower possesses 2 distinct vascular strands (pl. 1, *A*) in the ovary wall, the 3-stigma florets have 3 vascular strands in the ovary wall, and flowers having 5 stigmas have 2 ovaries, one with 3 and the other with 2 vascular elements (pl. 2, *B*).

CELL STRUCTURE OF STEM EPIDERMIS OF SEVERAL SUGARCANE × SORGO HYBRIDS AND THEIR PARENTS

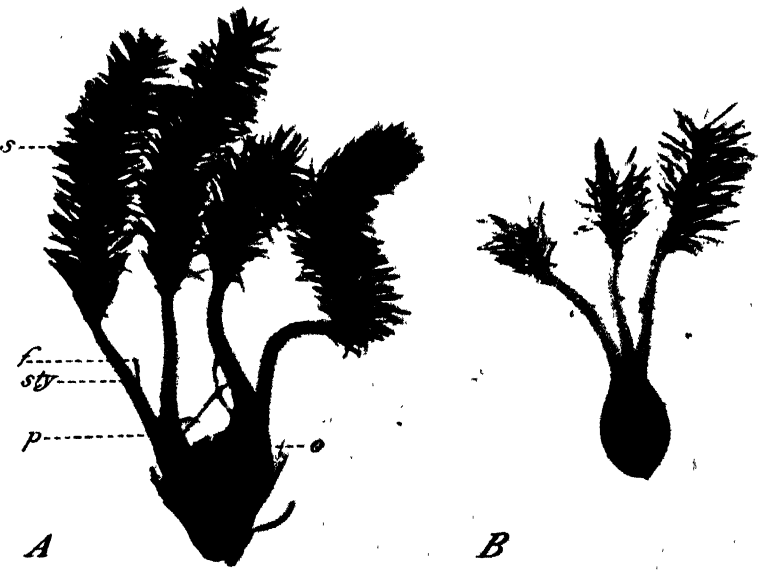
Owing to the great constancy of stem epidermal anatomical characters among different sugarcane varieties and their value for identification purposes (1), it was thought that a study of the influence of these parental characters on the hybrid progeny would prove of interest. Consequently, a detailed study was made of the stem epi-



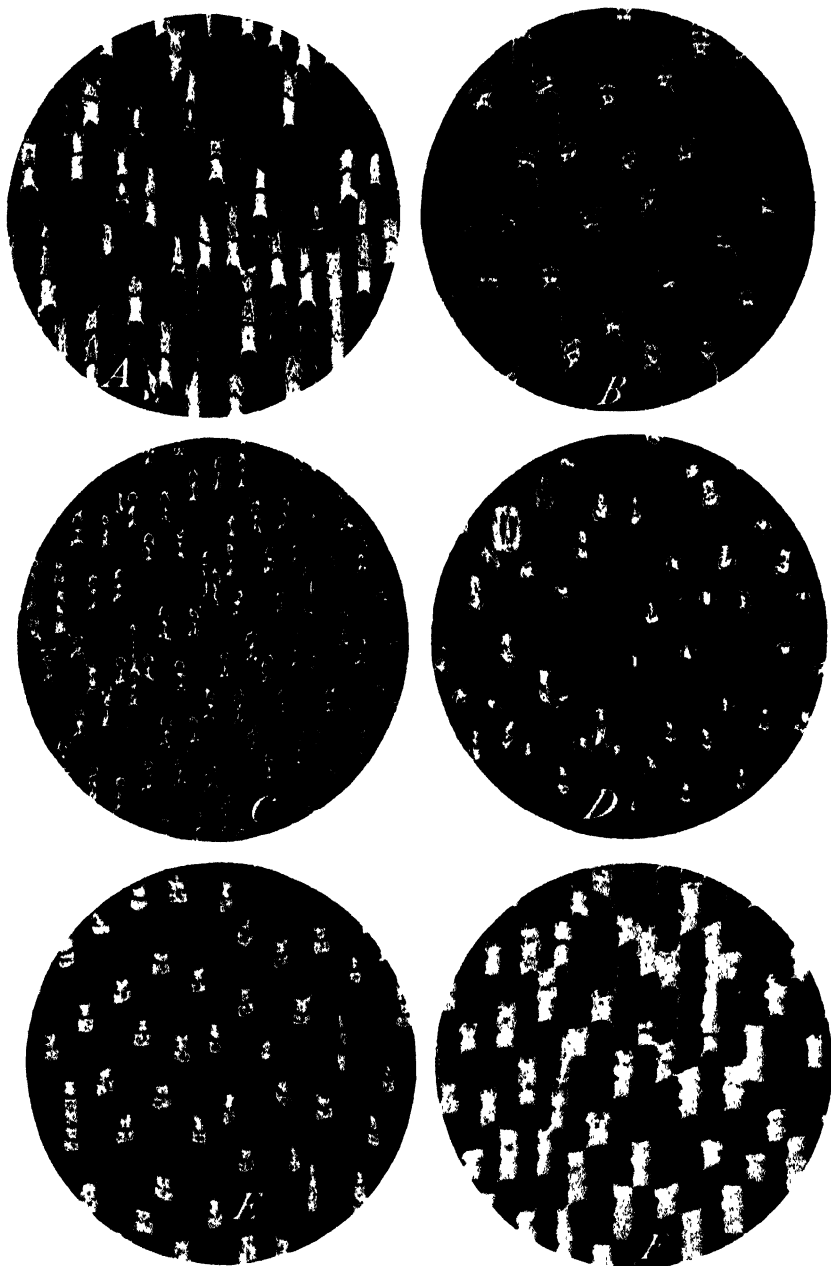
A, Cross section of normal spikelet of the hybrid F 31-13, showing *fl.*, Fertile lemma, *ig.*, inner glume; *st.*, sterile lemma, *og.*, outer glume; *p.*, palea; *lod.*, lodicule; *ov.*, ovary wall with two vascular strands; *o.*, ovule, and *st.*, stamen. $\times 105$ B, Cross section of spikelet of P. O. J. 2725 sugarcane, showing *lod.*, Lodicule, *ig.*, inner glume; *og.*, outer glume; *o.*, ovule; *ov.*, ovary wall; *stl.*, sterile lemma, and *p.*, palea $\times 105$. Note the greater diameter of the sugarcane \times sorgho hybrid spikelet as compared with that of sugarcane.



A, Portions of spikelet of F. 31-13, hybrid of P. O. J. 2725 sugarcane × Texas Seeded Ribbon sorgho, showing. *ogl*, Outer glume; *igl*, inner glume, *sl*, sterile lemma; *fl*, fertile lemma, *aw*, awn of fertile lemma. × 14
 B, Cross section through spikelet of the hybrid F. 31-13, showing two ovaries, one normal and the other aborted. This spikelet had five styles and stigmas. × 124.



1. Abnormal floret of the hybrid F 31-13 showing two ovaries, four styles and stigmas: *s*, Stigma, *f*, anther filament, *sty*, style, *p*, palea; and *o*, ovary. $\times 19$ B, Abnormal pistil of the hybrid F. 31-13 showing an ovary with three styles and stigmas $\times 19$



Anatomical structure of epidermis of stems of sugarcane, sorgo, and several hybrids between the two parental types. *A*, P. O. J. 2725 sugarcane. *B*, Texas Seeded Ribbon sorgo; *C*, hybrid F. 31-29; *D*, hybrid F. 31-33 (note frequent occurrence of solitary cork cells), *E*, hybrid F. 31-13; *F*, hybrid F. 31-6. All $\times 214$.

dermal anatomy of P. O. J. 2725 sugarcane, the two sorgo varieties, Texas Seeded Ribbon and Early (Kansas) Orange, and of several hybrids between each of the sorgo varieties and P. O. J. 2725 sugarcane, selected entirely at random. The results of this study are summarized in table 2.

TABLE 2.—*Epidermal characters of stem of sorgo, sugarcane, and several hybrids between these 2 grasses*

Variety ¹	Average width of cells	Short-cell groups per square millimeter	Stomata per microscopic field ²	Pointed elongated cork cells	Solidary cork cells	Solitary silica cells
	μ	Number	Number			
Texas Seeded Ribbon sorgo	12.1	545 ± 25	27.0 ± 0.10	Absent.....	Abundant..	Rare.
Early Orange sorgo	10.7	$1,033 \pm 27$	95.0 ± 9.1	do.....	Absent.....	Absent.
P. O. J. 2725 sugarcane	8.4	625 ± 24	2.8 ± 0.62	Very abundant	Very abundant	Rare.
F. 31-6	11.7	$1,320 \pm 11$	2.8 ± 0.9	Present.....	Rare.....	Do.
F. 31-10	10.3	800 ± 38	1 —	Scarce.....	do.....	Do.
F. 31-13	10.5	920 ± 00	5.5 ± 1.4	Absent.....	do.....	Do.
F. 31-29	10.4	$1,512 \pm 76$	36.0 ± 0.94	do.....	do.....	Absent.
F. 31-33	10.1	950 ± 31	13.4 ± 2.5	Scarce.....	Very abundant	Rare.
F. 31-40	11.2	$1,166 \pm 62$	5.2 ± 2.0	Absent.....	Rare.....	Do.
F. 31-61	10.6	850 ± 00	25.5 ± 0.35	Present.....	Abundant.....	Absent.

¹ F. 31-6, -10, and -13 are hybrids between Texas Seeded Ribbon sorgo (male) and P. O. J. 2725 sugarcane (female). Other F. 31 numbers are hybrids between Early (Kansas) Orange (male) and P. O. J. 2725 (female).

² Ocular $\times 10$ and 16-mm objective, or 1.9 mm ².

³ Standard error of the mean of not less than 5 determinations.

The average width of the cells of P. O. J. 2725 sugarcane (pl. 4, A) is unusually small (8.4 μ), in this respect resembling the sugarcane varieties D. 74 and Chunnee. The number of stomata (2.8 ± 0.62) per microscopic field is also small in this variety as compared with the number in the two sorgo varieties crossed with it, although such an occurrence is not at all unusual among sugarcane varieties. The great abundance of pointed, elongated cork cells in P. O. J. 2725 sugarcane and the complete absence of these in both of the sorgos, however, give contrasting characters of value for inheritance studies among the hybrid progeny.

The average widths of cells in the hybrid progeny F. 31-6 (pl. 4, F), F. 31-10, and F. 31-13 (pl. 4, E), crosses between P. O. J. 2725 sugarcane and Texas Seeded Ribbon sorgo, are all intermediate between those of the two parents. Similarly, the average widths of cells of the hybrid progeny F. 31-29 (pl. 4, C), F. 31-33 (pl. 4, D), and F. 31-61, crosses of P. O. J. 2725 sugarcane and Early Orange sorgo, are also intermediate between the two parents, F. 31-40 being the only one in this group with average cell width greater than that of Early Orange sorgo, the male parent.

In number of short-cell groups per square millimeter, all of the hybrids of both crosses were significantly greater than P. O. J. 2725 sugarcane, even though the Texas Seeded Ribbon sorgo parent (pl. 4, B) had less than P. O. J. 2725, the sugarcane parent. The fact that F. 31-6 had more than the sum of the two parents and F. 31-29 and F. 31-40 more than either parent, is also significant.

The number of stomata per microscopic field (1.9 mm²) is very high in both sorgo parents, especially in Early Orange. The average number of stomata per field among the four hybrids of P. O. J. 2725

sugarcane \times Early Orange sorgo is 20.0 ± 1.4 , while the average of the three hybrids of P. O. J. 2725 \times Texas Seeded Ribbon sorgo is only 3.1 ± 1.1 , showing the apparent dominance of the large number of stomata in Early Orange sorgo.

Pointed, elongated cork cells were very abundant in P. O. J. 2725 sugarcane and absent in both sorgo parents. These peculiar cells were never found very abundantly in the hybrids. More than half of the seven types examined had them, but in many cases they were rather scarce.

Although solitary cork cells are somewhat abundant in Texas Seeded Ribbon sorgo and very abundant in P. O. J. 2725 sugarcane, these cells occurred rarely in all three of the plants of this cross. On the other hand, although these solitary cork cells were absent in Early Orange sorgo, they were rather abundant in half of the hybrids with P. O. J. 2725 sugarcane, particularly in F. 31-33 (pl. 4, D).

The occurrence of solitary silica cells in the hybrids is in accordance with expectations. All the hybrids of P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo had them rarely, as did both parents, while in the case of hybrids of P. O. J. 2725 sugarcane \times Early Orange sorgo, which lacks these cells, 50 percent had none and in 50 percent they occurred rarely.

SUMMARY

A brief review is given of the successful efforts to secure sugarcane \times sorgo hybrids in Florida since 1930.

Some of the essentials for success in crossing sugarcane and sorgo in the Florida Everglades are pointed out; these are based on physiological studies of both sugarcane and sorgo pollen.

Of 345 hybrids produced thus far, only 128 have been raised to maturity. About 3 percent of these have shown enough vigor to warrant further field trials.

A comparative study has been made of the parents and hybrids as to leaf width, diameter of stem, height of plant, type of inflorescence and flower structure, when grown simultaneously under the same environment.

The hybrids of P. O. J. 2725 sugarcane \times Texas Seeded Ribbon sorgo were, on an average, intermediate between the parents in leaf width and stem diameter. The hybrid plants showed a wide variation in height, but the average height was significantly less than that of either parent.

The hybrid F. 31-13 showed several flower structures which were not found in either parent. The most conspicuous of these are an awned fertile lemma, 2 ovaries, and 3, 4, and even 5 styles and stigmas to the floret.

The average width of epidermal cells of the stems of hybrids resulting from crosses between P. O. J. 2725 sugarcane and Texas Seeded Ribbon sorgo and between P. O. J. 2725 sugarcane and Early Orange sorgo, is intermediate between those of the parents, in the majority of cases investigated.

All of the hybrids of both crosses investigated had a greater number of short-cell groups per square millimeter of the stem epidermis than the sugarcane parent P. O. J. 2725, even when the sorgo parent had fewer than the sugarcane with which it was crossed. One hybrid

had significantly more short-celled groups than the sum of the 2 parents, and 2 hybrids had more than either parent.

The average number of stem stomata per microscopic field of hybrids of P. O. J. 2725 sugarcane and Early Orange sorgo was significantly greater than that of hybrids of P. O. J. 2725 sugarcane and Texas Seeded Ribbon sorgo. This is attributed to the very large number of stem stomata per unit area in Early Orange sorgo.

Pointed, elongated cork cells were not abundant in the stem epidermis of any of the hybrids examined, these cells being absent in both sorgo parents but occurring very abundantly in P. O. J. 2725 sugarcane.

Solitary cork cells occurred rarely in the stem epidermis of all three of the hybrids of P. O. J. 2725 sugarcane × Texas Seeded Ribbon sorgo, although they were abundant in both parents. On the other hand, half of the hybrids of P. O. J. 2725 sugarcane × Early Orange sorgo had an abundance of solitary stem epidermal cork cells, although these cells were absent in Early Orange sorgo.

P. O. J. 2725 sugarcane, Texas Seeded Ribbon sorgo, and all the hybrids between these two parents rarely exhibited solitary silica cells in the stem epidermis. Fifty percent of the hybrids between P. O. J. 2725 sugarcane and Early Orange sorgo rarely showed solitary silica cells in the epidermis; the other 50 percent of the hybrids and the Early Orange sorgo parent lacked these cells entirely.

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THE EFFECT OF MAGNESIUM DEFICIENCY ON CROP PLANTS¹

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INTRODUCTION

In 1891 a field experiment was started at the Massachusetts Agricultural Experiment Station for the purpose of testing fertilizers for corn (*Zea mays* L.). From 1891 to 1929 the cropping system varied somewhat, but for most of this period it consisted of a short rotation of corn and grass; 2 years of corn followed by 2 years of mixed grasses. During this period plant nutrients were supplied solely by commercial fertilizers, and none of these carried appreciable amounts of magnesium. One-half the experimental field received lime in 1907 and again in 1921. No record was made of the magnesium content of the lime, although it was no doubt appreciable. The soil is a well-drained sandy loam with a gravelly subsoil of the Merrimac series. Variations in topography and mechanical composition combine to produce heterogeneous soil conditions which cause results in dry seasons considerably different from those in wet seasons.

Chlorosis was first observed in the leaves of corn grown on this field about 1920. By means of experiments conducted during the period 1924-28 Jones² proved that this chlorosis was due to a deficiency of available magnesium in the soil. In 1929 a new experiment was started on the same field for the purpose of determining the relative response of the more common crop plants to the deficiency of magnesium, and to observe and record the plant symptoms produced by this deficiency.

PROCEDURE

From 1929 to 1934, inclusive, 17 varieties of crops were grown. In addition, tobacco had been grown in the earlier period, and red clover and alfalfa were grown in pots filled with soil taken from the magnesium-deficient section of the experimental field.

Prior to 1929 the fertilizer treatments of the four plots into which the field was divided varied considerably. Details of these treatments were reported in an earlier publication.³ Beginning with 1929, the field was given annual applications of a 5-8-7⁴ fertilizer, but the rate at which this was used varied with the crop grown. Lime and magnesium treatments cut across the original fertilizer plots. The fertilizer, lime, and magnesium sulphate treatments for the period 1929-34 are given in table 1. The identity of the original plots was

¹ Received for publication Jan. 21, 1935; issued May 1935. Published as contribution no. 212 of the Massachusetts Agricultural Experiment Station

² JONES, J. P. DEFICIENCY OF MAGNESIUM THE CAUSE OF CHLOROSIS IN CORN. *Jour. Agr. Research* 39:873-892, illus. 1929.

³ JONES, J. P. See footnote 2.

⁴ Figures refer to the percentage of N, available P₂O₅, and water-soluble K₂O.

preserved, and it was found that previous fertilizer treatments affected the yield of crops during the last period. From 5 to 10 varieties of crops were grown on each of the original plots, thus giving four replicates⁵ of each variety. Individual plots varied from one three hundred and twentieth to one-eightieth acre in size. The original plots were given cross treatments of technically pure magnesium sulphate as shown in table 1. Field corn, variety Rustler's White Dent, which normally produces husking ears in this locality, was grown on each plot each year, and thus served as a standard for comparison with other crops. Counts of chlorotic corn plants were made each season.

TABLE 1.—Fertilizer, magnesium sulphate, and lime added to the soil from 1929 to 1934

Year	Crops	Fertilizer, ¹ per acre	Other materials
		<i>Pounds</i>	
1929	Corn	800	Magnesium sulphate, 400 pounds per acre to section C ² only.
	Timothy	500	
	Clover (alsike)	500	
	Potatoes	2,000	
	Onions	2,500	
1930	Corn	800	Do.
	Clover (alsike)	500	
	Timothy	500	
	Potatoes	2,000	
	Onions	2,500	
1931	Corn	800	No magnesium sulphate applied this year
	Clover (alsike)	500	
	Timothy	500	
	Potatoes	2,000	
	Onions	2,500	
1932	Soybeans	800	Magnesium sulphate, 400 pounds per acre to sections B and C, and 1 ton low-magnesium (1.29 percent MgO) ground limestone per acre to sections A and B in fall
	Clover (alsike)	500	
	Buckwheat	800	
	Soybeans	800	
	Corn	1,000	
	Oats	800	
	Oats	800	
1933 ³	Burley	800	No magnesium sulphate applied this year.
	Sudan grass	800	
	Japanese millet	800	
	Rutabagas	1,500	
	Corn	1,000	
	Buckwheat	800	
	Pigweed	800	
	Barley	800	
	Rye	800	
	Field corn	1,000	
1934 ⁴	Sweet corn	1,000	Magnesium sulphate, 100 pounds per acre applied to sections B and C.
	Japanese millet	800	
	Sudan grass	800	
	Rutabagas	1,600	
	Peppers	800	
	Mangels	1,600	
	Spinach	800	
	Turnips	800	

¹ 5-8-7 analysis.

² In the course of the experiment section A received lime; section B, lime and magnesium sulphate; section C, magnesium sulphate; section D, nothing except fertilizer which was applied to all sections.

³ Ammophos was used instead of superphosphate to supply P₂O₅ for 1933 and 1934.

⁴ Beginning with 1933, the east half of plot 1 was reserved for another experiment, thus reducing the number of replicates of some varieties to 2.

EXPERIMENTAL RESULTS

YIELDS

The figures for yield are given in table 2. The annual precipitation records for Amherst, Mass., for the period May to August, inclusive, are as follows:

	<i>Inches</i>		<i>Inches</i>
1929.....	9.47	1933.....	14.25
1930.....	14.13	1934.....	12.84
1931.....	22.12	Mean (1889-1928).....	15.74
1932.....	10.79		

Both season and variety were important factors in determining the results. The season of 1929 was abnormally dry and that of 1931 abnormally wet, each varying about 6.4 inches from the normal. These abnormalities depressed the actual yields. More significance should be attached to the corn crop than to any other because of its more frequent use in the experiment. The greatest relative effect of magnesium on the yield of grain corn was in 1931, a wet year, while one of the lowest relative yields of this crop was in the dry year 1929. The same order of agreement, however, does not hold for stover, but as Jones⁶ has pointed out the yield of grain apparently is more affected by a deficiency of magnesium than is the stover.

TABLE 2. - *Mean and relative yields of crops grown with fertilizer and added magnesium sulphate and lime, 1929 to 1934*

Crop	Years in which grown	Yield per acre (fertilizer only)	Relative yield ¹ with fertilizer plus—		
			Magnesium sulphate	Magnesium sulphate and lime	Lime
Corn, field (grain).....	1929 to 1934...	<i>Bushels</i> 42.5	95.0	100.7	99.2
Corn, field (stover).....	1929 to 1934	<i>Pounds</i> 2,147	99.7	107.8	112.2
Potatoes.....	1929 to 1931...	<i>Bushels</i> 204.3	105.7	106.3	97.0
Onions (from sets).....	1929, 1930.....	109.2	87.0	127.0	128.0
Onions (from seed).....	1931.....	70.3	89.0	111.0	118.0
Timothy.....	1930, 1931.....	<i>Pounds</i> 3,605	99.5	95.5	106.5
Clover (alsike).....	1930 to 1932.....	3,683	101.0	101.3	100.0
Soybeans (forage).....	1931, 1932.....	4,480	105.0	107.0	104.0
Buckwheat (grain and straw).....	1932, 1933.....	2,020	128.5	122.5	101.0
Oats (grain and straw).....	1932, 1933.....	4,262	102.5	95.0	97.0
Barley (grain and straw).....	1933, 1934.....	3,288	90.5	101.5	100.0
Sudan grass.....	1933, 1934.....	3,750	97.0	91.0	103.0
Millet (Japanese).....	1933, 1934.....	6,298	100.0	93.0	88.0
Rutabagas (early planting).....	1933, 1934.....	37,608	105.0	108.5	102.0
Rutabagas (late planting).....	1933.....	9,762	110.0	154.0	127.0
Corn, sweet (ears).....	1934.....	11,803	95.0	98.0	107.0
Corn, sweet (stover).....	1934.....	2,914	100.0	103.0	112.0
Mangels (roots).....	1934.....	85,507	118.0	114.0	100.0
Turnips (White Egg).....	1934.....	17,760	113.0	89.0	91.0
Spinach.....	1934.....	3,402	138.0	208.0	208.0
Peppers (fruit).....	1934.....	10,317	96.0	108.0	112.0
Peppers (plants).....	1934.....	655	93.0	100.0	103.0

¹ Yield with fertilizer only taken as 100.

² Dried in steam-heated oven to approximately 4 percent water. Work done in feed-control laboratory under supervision of P. H. Smith.

³ The comparatively low figure for the yield of spinach is due largely to the fact that the plants were more widely spaced than is usual in practice, and probably in part to a lower level of fertility than is common in vegetable gardening. However, it should be noted that yield figures are for dry matter rather than green weight.

⁶ Jones, J. P. (See footnote 2.)

A gravelly ridge running diagonally across the field is particularly effective in reducing yields of the magnesium-treated sections of plots 3 and 4 in dry seasons. This condition, together with a more favorable moisture relation of the checks, particularly of plot 3, is favorable to the checks in dry seasons. The variation in the yields from the different sections of the plots is shown by table 3. If plots 1, 2, and 4 are taken as more representative of uniform conditions, it will be seen that, except for the stover, the magnesium-treated sections were relatively better (e. g., 46.2 bushels *v.* 41.7 bushels) than the checks without lime. With lime this is true of plot 2 only. In the wet year of 1931 the yield of the magnesium-treated section of plot 1 exceeded that of the check by 27.9 percent for both grain and stover.

The effect of season on the absorption of available magnesium is well shown by the data on amount of chlorosis given in table 4. Here it will be noted that the highest percentage of chlorotic plants occurred in the wet year 1931. By comparing table 4 with the precipitation data it will be seen that there is almost perfect agreement between precipitation and extent of chlorosis in corn.

TABLE 3.—Mean yield of corn, grain, and stover, as affected by additions of magnesium sulphate and lime, by plot sections, 1929-34

Crop and plot	Yield on plots treated with fertilizer plus—			
	Nothing	Magne- sium sulphate	Magne- sium sul- phate and lime	Lime
Corn (grain):	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>
Plot 1.....	41.1	44.0	44.7	50.0
Plot 2.....	41.7	46.2	45.9	30.6
Plot 3.....	51.2	29.8	37.3	42.6
Plot 4.....	36.1	41.7	43.3	45.6
Corn (stover):	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Plot 1.....	2,101	2,501	2,617	2,744
Plot 2.....	2,166	2,156	2,443	2,090
Plot 3.....	2,589	2,135	2,313	2,526
Plot 4.....	2,032	2,205	2,368	2,625

TABLE 4.—Percentage of normal and chlorotic corn plants as influenced by addition of magnesium sulphate and lime, 1929-34

Year	Treatment in addition to fertilizer	Plants that were—				Year	Treatment in addition to fertilizer	Plants that were—			
		Nor- mal	Chlorotic		Nor- mal			Chlorotic			
			Slightly	Strongly				Slightly	Strongly		
		Pct.	Pct.	Pct.			Pct.	Pct.	Pct.		
1929..	None.....	39	18	43	1932..	None.....	16	45	39		
	Magnesium sulphate.....	87	9	4		Magnesium sulphate.....	100	0	0		
	Magnesium sulphate and lime.....	85	9	6		Magnesium sulphate and lime.....	100	0	0		
	Lime.....	84	9	7		Lime.....	79	19	2		
1930..	None.....	35	54	11	1933..	None.....	9	37	54		
	Magnesium sulphate.....	98	2	0		Magnesium sulphate.....	100	0	0		
	Magnesium sulphate and lime.....	96	4	0		Magnesium sulphate and lime.....	98	2	0		
	Lime.....	79	20	1		Lime.....	70	25	5		
1931..	None.....	5	40	55	1934..	None.....	20	46	29		
	Magnesium sulphate.....	85	14	1		Magnesium sulphate.....	100	0	0		
	Magnesium sulphate and lime.....	78	20	2		Magnesium sulphate and lime.....	100	0	0		
	Lime.....	39	52	9		Lime.....	95	4	1		

Of all crops grown, buckwheat and spinach were most affected by a deficiency of magnesium, but as there are only 1 year's results for spinach, they must be considered as indicative only.⁷ The yield of buckwheat was not only considerably increased by magnesium, but the physiological symptoms also were marked. This crop appears to be the most sensitive to magnesium deficiency of all those studied in this experiment. It will be noted that the beneficial effects of magnesium are enhanced by the addition of lime.

PHYSIOLOGICAL SYMPTOMS

One of the principal objectives of this work was the determination of the plant symptoms associated with magnesium deficiency. Con-

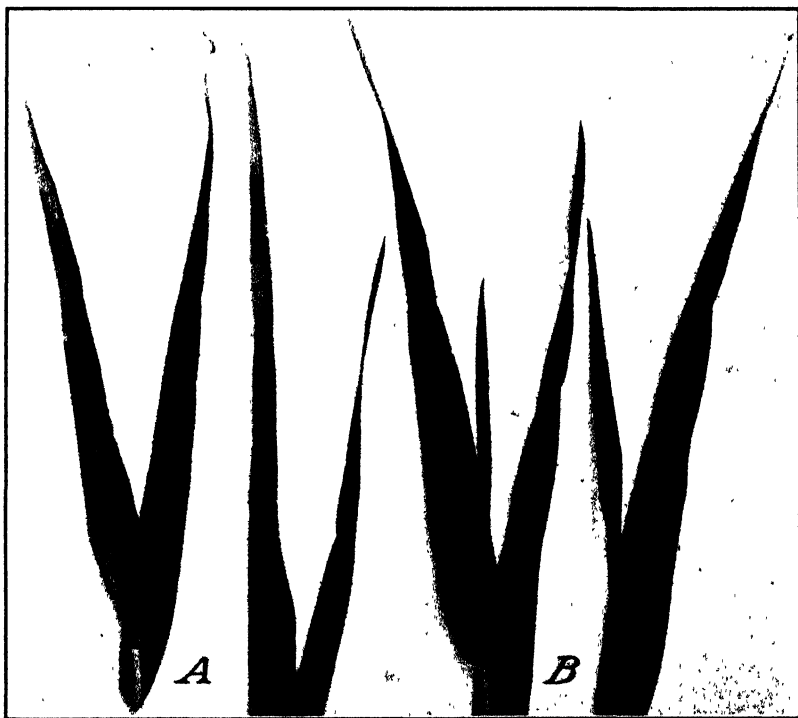


FIGURE 1 Barley leaves A, Mildly chlorotic, striped type, from magnesium-deficient soil, B, normal, from soil treated with magnesium sulphate.

siderable difference in the effect of the deficiency was noted among the crops grown. The more sensitive species developed symptoms which varied from a mild chlorosis to extreme necrosis of the leaf tissue.

Jones, in a previous report⁸ on this work, accurately described the symptoms of this physiological disease as it affects corn, and Garner and others⁹ have described its effects on tobacco. Nothing needs to be added to Jones' description of the symptoms on corn

⁷ Pot experiments in the greenhouse appeared to corroborate the field observations on spinach.

⁸ JONES, J. P. See footnote 2.

⁹ GARNER, W. W.; McMURTREY, J. E.; BACON, C. W., and MOSS, E. G. SAND DROWN, A CHLOROSIS OF TOBACCO DUE TO MAGNESIUM DEFICIENCY, AND THE RELATION OF SULPHATES AND CHLORIDES OF POTASSIUM TO THE DISEASE. *Jour. Agr. Research* 23:27-40, illus. 1923.

except to state that incipient chlorosis sometimes appeared first on the margin and tip of the leaves instead of in the intervascular tissue. On other soils having a severer deficiency of magnesium it has been observed that corn leaves crinkle and droop considerably in advanced stages of the disease.

The pattern of chlorosis developed by other plants followed rather closely the type of venation of the leaves. Leaves having parallel veins, such as certain small grains and grasses, developed the same type of chlorosis as corn, but of a mild type (fig. 1). Oats showed the symptoms in an early stage of growth, but they later disappeared. It should be stated that some parallel-veined plants have leaves that normally are more or less striped, or else the striping is due to some deficiency other than magnesium. Such was the case with Japanese millet. The effect of magnesium deficiency on the different plants used in the experiment is shown in table 5.

TABLE 5.—*Plant symptoms produced by a moderate magnesium deficiency in the soil*

Plant	Chlorosis			Remarks	Plants	Chlorosis			Remarks
	None	Mild	Strong			None	Mild	Strong	
Corn (field).....		X	X	Striped, necrotic. Do.	Barley		X		Striped
Corn (sweet).....		X	X		Sudan grass		X		Do.
Potato.....	X	X		Marginal, mottled.	Millet (Japanese).....	X			Mottled. Do. Do.
Onion.....	X	X			Rutabaga.....		X	X	
Timothy.....	X	X			Mangel.....		X		
Clover (alsike).....	X	X			Turnip (White Egg).....		X	X	Do.
Clover (red) ¹	X	X			Spinach.....		X	X	Sunscald necrosis.
Alfalfa ¹	X			Mottled, necrotic When young, only.	Pepper.....		X		Mottled Do.
Soybean.....		X			Tobacco.....		X	X	
Buckwheat.....			X						
Oat.....		X							

¹ Pot experiment.

Susceptible plants with leaves of netted venation as a rule developed a mottled pattern similar to the well-known appearance of tobacco leaves affected by "sand drown." Buckwheat and turnips (and tobacco in the earlier experiment on this soil) were the outstanding plants which showed this type of chlorosis (figs. 2 and 3). With these plants, also, incipient chlorosis often appeared in the leaf margin instead of in the intervascular tissue. In advanced stages of the disease cupping or curling of the leaf margin was common, the tissue turned yellow or brown, and the leaves dropped from the plant. A necrosis of intervascular tissue similar to sunscald often occurred, particularly in leaves exposed to full action of the sun's rays.

Spinach was affected differently. The typical chlorosis shown by other plants did not appear, but instead marked necrotic areas developed in the intervascular tissue. These areas were whitish and of a papery texture, and similar to sunscald areas (fig. 4). They appeared within a very short time after conditions were right. It is thought that lesions of this kind might be expected in the mesophyllic tissue of a leaf of a rapidly growing plant like spinach if the tissue were deficient in some essential ingredient. Apparently this tissue in its condition of rapid growth and tenderness skipped the usual

chlorotic stage or passed through it so rapidly that it was not observed. The observations of only 1 year are available on this crop and conclusions can, therefore, be only tentative.

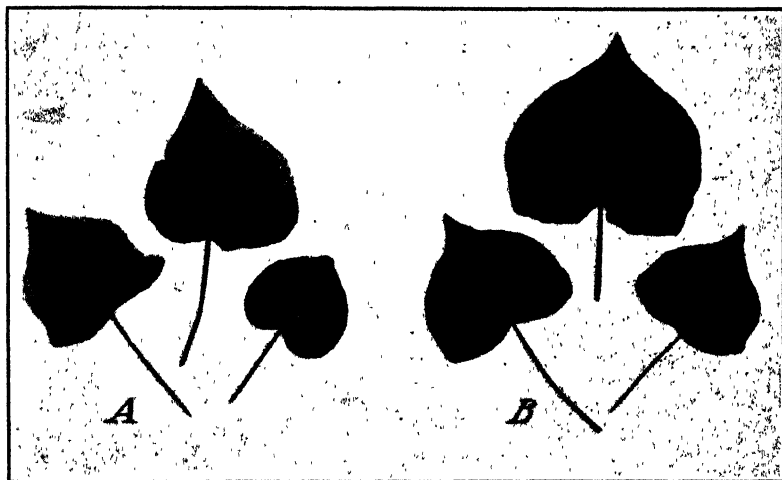


FIGURE 2 —Buckwheat leaves: *A*, Strongly chlorotic and necrotic, mottled type of chlorosis, from magnesium-deficient soil; *B*, normal, from soil treated with magnesium sulphate.

A characteristic symptom of magnesium deficiency observed in these experiments is that the chlorosis appeared first on the older



FIGURE 3.—Turnip leaves: *A*, Strongly chlorotic and necrotic, mottled type of chlorosis, from magnesium-deficient soil; *B*, normal, from soil treated with magnesium sulphate.

leaves, regardless of the type of plant. This characteristic should serve to distinguish the chlorosis due to magnesium deficiency from that due to some other causes.

EFFECT OF MAGNESIUM DEFICIENCY ON CHEMICAL COMPOSITION OF PLANTS

The effect of magnesium deficiency in this soil on the mineral constituents of different parts of the corn plant has been reported by Jones.¹⁰ His data showed that the content of magnesium in the plant corresponded to the application of magnesium sulphate to the soil. Work along this line has been continued in the present studies. In table 6 are given analytical results for the years 1930, 1931, and 1933. The rainfall during the growing season of 1931 was unusually

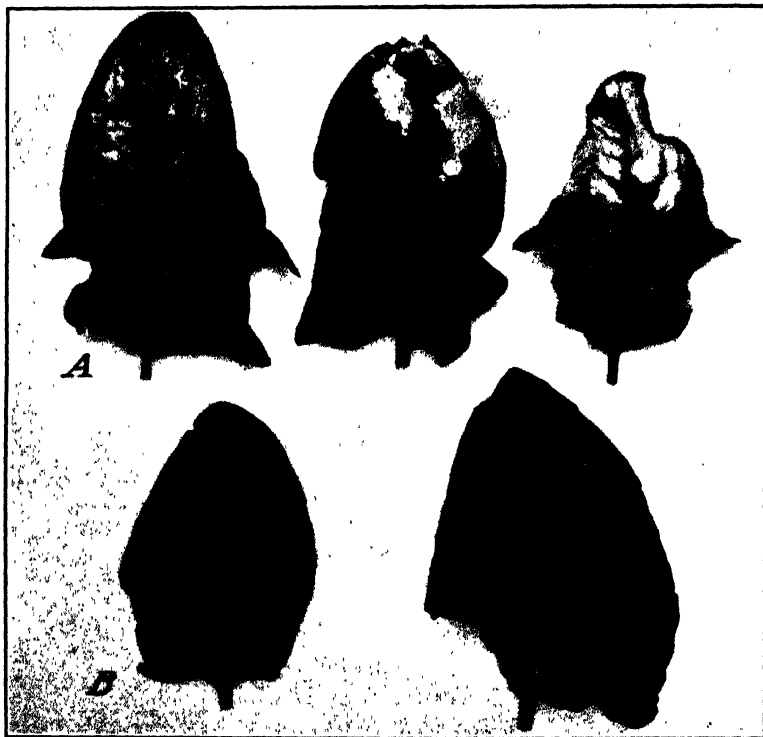


FIGURE 4.—Spinach leaves: A, Necrotic, from magnesium-deficient soil; B, normal, from soil treated with magnesium sulphate.

high, 6.4 inches above normal, while that of the other 2 years was a little below normal. This difference in precipitation is reflected in the magnesium content of the corn stover, which was lowest in the wet year of 1931. The data as a whole show that the magnesium content of the crop was enhanced by applications of magnesium sulphate to the soil. This increase was greatest in those crops, such as buckwheat, that showed the greatest response to magnesium in yield and symptoms.

¹⁰ JONES, J. P. (See footnote 2.)

TABLE 6.—*Percentage magnesium oxide and calcium oxide in dry matter of several crops grown with fertilizer only and with fertilizer and added magnesium sulphate and limestone*¹

Year and crop	Analysis of plants from plots treated with fertilizer plus—							
	Nothing		Magnesium sulphate		Magnesium sulphate and lime		Lime	
	MgO	CaO	MgO	CaO	MgO	CaO	MgO	CaO
1930 ²	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Corn stover.....	0.183	0.603	0.338	0.580	0.315	0.605	0.228	0.625
1931 ²								
Corn stover.....	.143	.605	.308	.523	.268	.585	.206	.730
1933 ³								
Corn and cob.....	.151	.139	.247	.138	.187	.127	.187	.145
Corn stover.....	.229	.634	.395	.541	.372	.568	.299	.692
Oats (grain and straw).....	.518	.680	.604	.614	.679	.690	.529	.715
Barley (grain and straw).....	.427	.733	.595	.719	.657	.668	.436	.805
Sudan grass709	1.150	.699	1.200	.537	1.129	.511	1.134
Millet.....	.608	1.311	.769	.990	.694	1.528	.618	1.330
Buckwheat (grain and straw).....	.442	3.360	.728	2.468	.592	3.075	.475	4.064
Rutabagas (roots).....	.308	.794	.245	.979	.205	.776	.181	.961
Rutabagas (tops).....	.289	3.409	.466	3.071	.394	3.935	.331	4.081

¹ Chemical work was done in the fertilizer control laboratory under direction of H. D. Haskins.² Mean of analyses of samples from 4 replicated plots, 1930 and 1931.³ In 1933 analyses were of composited samples from 4 replicates.

GENERAL DISCUSSION

Observations and studies recently made of other magnesium-deficient soils in the Connecticut Valley indicate that the soil on which these experiments were conducted is only moderately deficient in magnesium. Many more extreme cases have been observed, and these were on soils containing somewhat less of the finer separates and often much more gravel. Magnesium deficiency on such soils has been found to be associated with moderate to strong acidity, and no doubt much of the trouble with crop growth was due to strong acidity or calcium deficiency, or both.

The unlimed half (secs. C and D) of the field used for these experiments has a reaction of pH 5.6 and the limed half pH 6.4. Some Connecticut Valley soils showing extreme magnesium deficiency have tested about pH 4.0. Some of these extreme cases have shown also a much greater magnesium deficiency by the rapid colorimetric field tests than the soil of the field used in these experiments. The surface soil of this field was found to contain from 8.7 to 20.3 parts per million of magnesium in the untreated section, by extraction with potassium chloride,¹¹ and these figures agree fairly closely with the estimates by the rapid semiquantitative methods. The untreated surface soil contained 0.747 percent total magnesium. It appears that a soil should contain from 30 to 40 parts per million of easily replaceable magnesium, or 60 to 80 pounds per acre, to avoid magnesium deficiency.

¹¹ HADDOCK, J. L. STUDIES OF METHODS FOR DETERMINATION OF MAGNESIUM DEFICIENCY IN SOILS. 1932. (Unpublished thesis.)

SUMMARY

The crops grown differed to a marked degree in their response to a deficiency of magnesium in the soil. Buckwheat and spinach were most affected, and turnips, mangels, corn, and tobacco considerably so. The small grains, grasses, clovers, and potatoes were only slightly affected, and other plants not at all. Plants sensitive to magnesium deficiency developed characteristic physiological symptoms which have value in diagnosis. Chlorosis of the older leaves developed in the intervascular tissue. In the leaves of plants with parallel veins this produced a striped appearance, while in the leaves having netted venation, a mottled pattern was produced. In severe cases of chlorosis the margin or the entire leaf turned brown, and in some cases the leaf dropped from the plant.

The addition of magnesium sulphate to the soil increased the percentage of magnesium in the plant or portions of it. The increase was greatest in those crops whose yields and appearance were most affected by the deficiency of magnesium. The content of magnesium in the plant was affected also by the amount of precipitation during the growing season, being less in seasons of heavy rainfall.

THE SUSCEPTIBILITY OF FLOWER BUDS OF THE MONTMORENCY CHERRY TO INJURY FROM LOW TEMPERATURE ¹

By V. R. GARDNER

Director, Michigan Agricultural Experiment Station

INTRODUCTION

Injury to flower buds or developing flowers from low temperatures has long been recognized as in some degree a limiting factor in sour-cherry production. Nevertheless, most varieties of sour cherry (*Prunus cerasus* L.) are regarded as comparatively hardy, their flower buds being as resistant to cold in midwinter as those of the apple, though in the preblossoming stage of early spring they become more tender than those of the peach. Goff ² reported that a comparatively large percentage of the flower buds of most varieties survived a minimum temperature of -27.5° F. at Madison, Wis., in 1899, though somewhat more serious injury was recorded following the milder winter (-23° minimum) of 1896-97, which had been preceded by a rather dry summer. Temperatures ranging from -22° to -37° in February 1934, at various points in northern Michigan where the Montmorency is extensively grown, killed from 5 to 20 percent of the buds (as determined by random sampling), hardly enough greatly to reduce the size of the following crop. On the other hand, data collected by the Michigan Agricultural Experiment Station during recent years in connection with its study of bud variation indicate that the number of flower buds killed at comparatively low temperatures is sometimes smaller than at other times when the temperature is somewhat higher, and that there is much variation between trees in the same orchard in susceptibility to injury of this kind. Records bearing on certain aspects of this question are presented in this paper.

KILLING OF DORMANT FLOWER BUDS BY LOW TEMPERATURES

Besides winter-killing records on certain individual Montmorency trees, selected because of apparent marked susceptibility or resistance of the flower buds to winter cold, counts were made on large random samples of buds from each of 149 trees in a block on the grounds of the Graham Horticultural Experiment Station at Grand Rapids, Mich., following the winters of 1932-33 and 1933-34, and in the so-called "Corporation" orchard of 190 trees near South Haven, Mich., for the same years. Minimum temperatures of -10° F. and -16° were recorded on February 9 of both years at Grand Rapids and -10° and -17° on the same dates at South Haven.

Killing of individual flowers in the buds ranged from 0 to 5 percent in the winter of 1932-33 at Grand Rapids and from 2 to 37 percent in the winter of 1933-34. Averages for the 2 years were 0.7 and 11.5

¹ Received for publication Nov. 20, 1934; issued May 1935. Journal article no. 196 (N. S.) from the Michigan Agricultural Experiment Station.

² Goff, E. S. THE DEGREE OF COLD ENDURABLE BY FLOWER-BUDS OF THE PLUM AND CHERRY. Wis. Agr. Expt. Sta. Ann. Rept. 14: 309-313, illus., 1897.

percent. At South Haven killing of individual flowers in the bud ranged from 0 to 22 percent in the winter of 1932-33 and from 0 to 21 percent in the winter of 1933-34. Averages for the 2 years were 5.7 and 5.6 percent. The period covered by these figures is too short to establish them as an accurate measure of the extent to which the dormant flower buds of this variety are winter-killed, but when they are considered in connection with observations extending over many years the conclusion seems warranted that bud killing of this type is not an important limiting factor in the production of Montmorency cherries in Michigan. Doubtless, killing of individual flowers in excess of 20 percent is likely to lead to some reduction in yield, though the higher percentages of blossoms that set fruit under such conditions usually compensate in part for the reduction in flower number. A com-

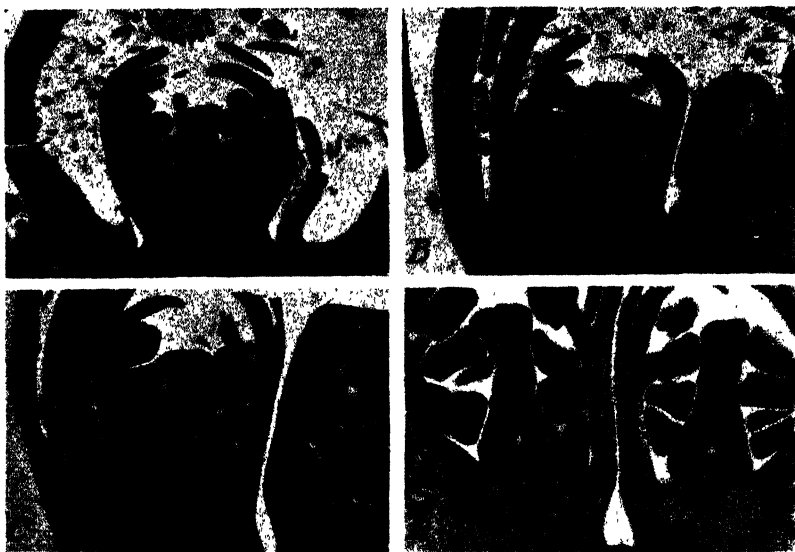


FIGURE 1.—Photomicrographs showing representative flower-bud development in a sport of the Montmorency cherry whose buds are especially tender to low winter and spring temperatures, compared with normal buds: A and C, Buds of Selection 159, which is tender to cold, collected November 2, 1932, and March 28, 1933; B and D, buds from adjacent normal Montmorency trees collected the same dates. $\times 33$.

parison of injury in individual trees for 2 years is possible only from the records of the Corporation orchard. Only a few trees whose buds were injured rather severely one winter showed a high degree of injury the next winter. However, this is not surprising in view of the relatively small differences between these trees.

Buds from a number of trees in both the Graham Station and Corporation orchards which showed some of the most and some of the least winter-killing were collected in late fall and again in early spring for sectioning, to determine whether such differences as were found in resistance to low winter temperatures might be correlated with differences in state of advancement. The flower buds themselves, their rudimentary pistils, and their other flower parts varied more or less in size from tree to tree when collected, say, November 1 or March 15, but the degree of differentiation attained at any particular date in any 1 year varied very little between trees in a single locality.

Furthermore, the slight variations above or below the average in size and in degree of differentiation were about equally distributed among trees with the more hardy and those with the less hardy buds. A study of the buds of a Montmorency tree in an orchard near So-lus, Mich., planted in 1904 and always semibarren, furnishes additional evidence on this latter point. This tree, to be regarded as a bud sport,³ forms an abundance of flower buds that at the close of the growing season cannot be distinguished from those of surrounding, normally productive trees. Nor can the buds which survive the winter be distinguished in early spring from those on adjacent trees.

However, about two-thirds of the buds are regularly winter-killed. Their tenderness is due to or associated with some physiological condition rather than with any peculiarity of structure. This is well brought out in figures 1 and 2, which show the flower parts of average



FIGURE 2 — Representative flower buds of a sport of the Montmorency cherry whose buds are especially tender to low winter and spring temperatures, compared with normal buds: A, Buds of Selection 159, which is tender to cold and one of whose flowers had been killed by frost 3 days previous to collection on April 29, 1933; B, buds of an adjacent Montmorency tree more resistant to low temperatures, collected the same date. $\times 7$.

flower buds from this winter-tender tree, along with those from adjacent normal trees, collected at intervals.

DELAYED WINTER-KILLING OF FLOWER BUDS BY LOW TEMPERATURES

Montmorency buds in which all of the individual flowers have been killed during the dormant season rarely enlarge in the spring, and their bud scales do not separate even at the tip. As the season advances they dry out, an abscission layer forms at their bases, and by the middle or end of the blossoming season most of them have fallen off. Buds in which some of the individual flower buds have been killed but in which one or more have survived enlarge and open very much like those in which none of the individual flowers has been harmed. Many buds enlarge to several times their winter size, reflex their outer scales, and expand their inner scales without, however, protruding any blossoms. The exposed outer surfaces of the inner bud scales acquire a purplish pink color. Finally, at about the full-bloom stage these buds that several weeks earlier gave promise

³ The term "bud sport" is used in this article to refer to a whole-tree or limb variant that has been under observation for a number of years so that the permanence of its deviation from type in the orchard or in the tree is established. In most of the instances cited the variant and the accompanying parent or normal form have been propagated vegetatively, but in only a few instances have the daughter trees been under observation long enough to permit a comparison of their behavior with that of the parent forms.

of producing a full crop of flowers, fall off. Examination shows that the bud that behaves in this way survived the winter and resumed growth and differentiation in the spring. As growth is resumed, however, the developing flower buds become relatively susceptible



FIGURE 3.—A, A spur showing 2 flower buds (*aa*) killed during the dormant season and 2 in each of which 2 individual flowers were uninjured; B, C, 2 twigs showing flower buds that had all or part of their individual flowers killed by the late type of winter-killing; D, 1 flower bud (*a*) on this shoot had all its individual flowers killed while dormant, 2 buds (*b*) suffered no winter-killing of either the dormant season or late type, and the others had all or part of their individual flowers killed by the late type of winter-killing. Photo taken May 15, 1934.

to injury from low temperatures, and observations indicate that this is a far more critical period than the dormant season. The data indicate further that there are wide variations between different trees in the same orchard growing under the same conditions, and also between different limbs of the same tree, in the susceptibility of their

flower buds to killing at this stage. The difference between this late form of winter-killing of flower buds and that which occurs while the buds are dormant is shown in figure 3, and the difference in stage of development of the individual flowers and flower parts at the time the injuries occur is shown in figure 4.

Sharp demarcation of dormant-season killing from killing associated with early growth resumption cannot be made. This latter, in turn, merges into still later killings of buds near or at the blossoming stage. However, the failure of the early killed buds to swell in the one case



FIGURE 4.—Sections of flower buds of the Montmorency cherry showing killing from midwinter freezing (A) and killing of the late winter type (B). Both sections are of buds collected May 15 when the trees were in full bloom. $\times 13$.

and of the individual flower buds to protrude beyond the separating bud scales in the other fairly accurately delimits what is here described as delayed winter-killing. Assignment of this type of injury to winter or to spring is largely a matter of judgment, but it occurs at a stage when advancement from dormancy is so slight that it may be regarded as a type of winter-killing. So far as the writer is aware, this type of winter-killing has not been described or recognized in the literature dealing with the sour cherry.

Extensive quantitative data are not available to show the amount of this form of bud killing in different sections or in different years. That it may sometimes be serious, however, is indicated by the fact that in a random sample of flower buds from one large Montmorency

tree in an orchard near Traverse City, Mich., examined May 15, 1934, all of the individual flowers were dead in 71 percent of the buds and about 70 percent of the blossoms in the remaining 29 percent; that is, the total blossom killing amounted to over 90 percent. Many other trees in the same orchard suffered serious injury of the same type, though none quite so heavily. Observations in many Montmorency cherry orchards of Michigan during the past 10 years lead to the opinion that in general between 5 and 10 times as many flower buds are killed while in this early post-dormant stage as are killed by much lower temperatures in the dormant stage. Unquestionably delayed winter-killing is a factor of considerable importance in limiting yield in the Montmorency cherry orchards of Michigan, though it is less serious than spring frost injury, which comes still later.

KILLING OF FLOWER BUDS BY SPRING FROSTS

Every cherry grower recognizes the danger of the serious reduction or perhaps entire loss of his crop from the killing of fully opened blossoms or of unopened blossom buds by spring frosts. Indeed, this is generally considered the greatest hazard in sour cherry production, even more of a hazard than it is with most other deciduous fruits because of the apparent greater susceptibility of the flowers to frost injury. Some measure of its importance may be obtained from records collected for the 149 Montmorency trees at the Graham Station for the years 1932-34. In the spring of 1932 the percentage of blossoms or blossom buds killed by spring frost on different trees ranged from 32 to 99, with an average of 77; in the spring of 1933 the range was from 20 to 90, with an average of 60; and in the spring of 1934 the range was from 1 to 95, with an average of 25. In the Corporation orchard killing by spring frosts on the 190 trees ranged from 4 to 100 percent in 1931, with an average of 56; from 25 to 97 percent in 1933, with an average of 73. There was no frost injury in this orchard in 1932 or 1934. In some orchards the loss would be distinctly smaller, in others much greater.

Records for individual trees in these two and in a number of other orchards show clearly that, though some trees present considerable variation in the relative amount of frost injury from year to year, many others are remarkably consistent in the reaction to cold of their open flowers or unopened flower buds. This is well brought out in tables 1 and 2.

TABLE 1.—Percentages of blossoms killed by frost in the Graham Station and the Corporation orchards, 1931-34

Orchard and trees	Percentage of blossoms killed in—			
	1931	1932	1933	1934
Graham Station:				
All trees		77	60	25
25 trees most injured in 1932		94	64	25
25 trees most injured in 1933		52	80	19
25 trees most injured in 1934		80	60	68
25 trees least injured in 1932		54	49	16
25 trees least injured in 1933		63	40	21
25 trees least injured in 1934		76	57	2
Corporation:				
All trees	55.8	0	73.0	0
25 trees most injured in 1931	98.0	0	85.0	0
25 trees most injured in 1933	81.0	0	92.7	0
25 trees least injured in 1931	10.9	0	61.0	0
25 trees least injured in 1933	32.0	0	46.0	0

TABLE 2.—Records of spring frost injury to selected trees in the Graham Station and the Corporation orchards, 1931–34

Orchard	Tree no.	Percentage of blossoms killed in—			
		1931	1932	1933	1934
Graham Station.....	5B	()	49	41	4
	5H	()	55	45	1
	3J	()	63	38	3
	2E	()	90	58	92
	4E	()	83	55	94
	9E	()	94	70	93
Corporation.....	21	8	0	39	0
	47	8	0	37	0
	137	14	0	25	0
	59	100	0	94	0
	125	95	0	97	0
	144	95	0	95	0

† Undetermined.

In view of the fact that as cherries approach the blossoming stage more or less unevenness in degree of development of the flower buds

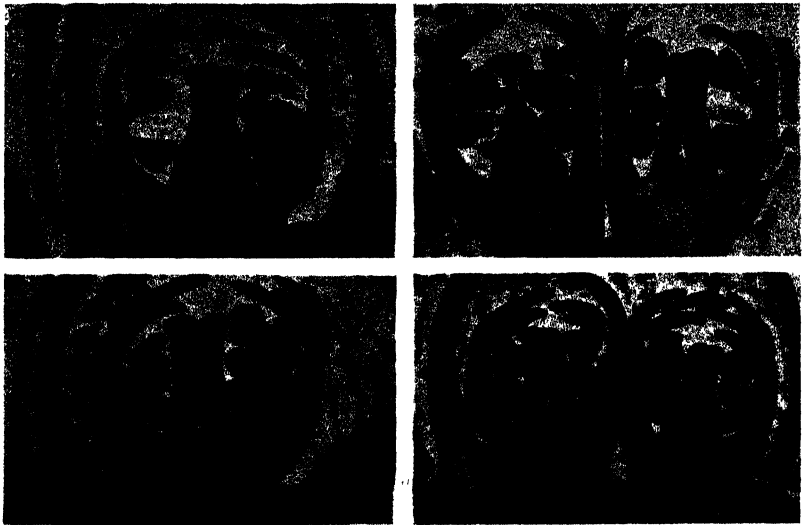


FIGURE 5. —A and B, photomicrographs showing development of representative flower buds of selection 516, a tree normally resistant to injury from midwinter cold and from spring frost, collected November 9, 1932, and March 29, 1933, respectively; C and D are photomicrographs showing development of representative flower buds of selection 517, a bud sport in tree no. 516, the buds of which are relatively susceptible to injury from midwinter cold and spring frost, collected November 9, 1932, and March 29, 1933, respectively. Note that the buds of selection 517 are practically indistinguishable from those of selection 516. $\times 33$.

is always in evidence, the question naturally arises whether the more advanced or the less advanced flowers suffer most from a damaging frost. The answer is that, in general, when flower buds are one-quarter to one-half the size they attain just before they open they appear to be slightly but distinctly more sensitive to frost injury than when they are fully developed and ready to open or when the flower is partly open or fully expanded, though this may be associated with the fact that the frosts occurring during the earlier stages of development are usually more severe than those occurring a few days later.

Large numbers of field records, however, warrant the definite statement that the differences in susceptibility from tree to tree are so great and the differences in advancement so small, that direct connection between them seems improbable.

Examinations were made of sections of random samples of large numbers of flower buds from a considerable number of individual



FIGURE 6.—Flower bud of the relatively frost-resistant normal form (selection 516) of the Montmorency cherry (A) and of a bud sport (selection 517) of the same tree (B) the buds of which are relatively tender to frost. Material collected for sectioning April 29, 1933, 3 days after the frost occurred; 1 injured pistil in A. Note that there is relatively little difference in stage of development between the frost-resistant and frost-susceptible buds though there is some difference in sizes of flower parts. $\times 10$.

Montmorency trees and of their limb sports, collected at various periods (e. g., June 30, July 15, Aug. 15, Nov. 1, late March, mid-April, late April). The results showed that in the relatively late blossoming trees or limb sports and in those that blossom rather early, in those whose flower buds are relatively susceptible and in those whose flower buds are very resistant to spring frost injury



FIGURE 7.—Representative flower buds of selection 365, a frost-tender but late-blossoming sport of the Montmorency cherry. Comparison of the March 28 collection A ($\times 33$) with B and C in figure 5 show that the buds of this sport had reached a normal stage of development in late winter. Comparison of the April 29 collection B ($\times 10$) with A and B in figure 6 indicates a slower development in early spring.

(1) the flower buds are differentiated at practically the same time of the year, (2) the buds attain essentially the same stage of differentiation at the beginning of the dormant season, (3) bud growth is resumed at practically the same time in late winter or early spring, (4) initial growth rate in the spring is essentially the same, but (5) as the growing season advances differences in rate of development become increasingly pronounced. These statements are supported by the photomicrographs shown in figures 5, 6, and 7, selected as

fairly representative of many thousands of buds that were examined. The evidence, therefore, indicates clearly that the major differences in susceptibility to spring frost are of a nature not revealed by anatomical study. It also indicates that cultural methods intended to hasten or delay flower-bud differentiation or advancement, do not afford a very promising approach to the solution of the frost-injury problem in the Montmorency cherry although in Wisconsin some relationship between early differentiation and advanced fall development on the one hand and susceptibility to low winter temperatures on the other has been reported.⁴

CORRELATION BETWEEN THE DIFFERENT TYPES OF INJURY TO FLOWER BUDS CAUSED BY LOW TEMPERATURES

The question arises whether a whole tree bud sport or a limb sport whose flower buds are markedly resistant or markedly susceptible to one form of injury is likewise markedly hardy or markedly tender to another form. The data collected indicate that a sport whose flower buds are, say, especially susceptible to winter-killing while in the dormant state are neither more nor less likely to be susceptible to late winter-killing or to spring frost injury. For instance, on selection 365, a whole-tree variant in the Corporation orchard, the flower buds were killed to the extent of 100 and 93 percent in late April of 1931 and 1933, but none of its flower buds were killed by mid-winter freezing in 1932-33 and only 2.5 percent were killed during the much severer winter of 1933-34. At no time during the 1930-34 period did the flower buds on this tree show an appreciable amount of the late form of winter-killing. Selection 420, a whole-tree variant in the Titus orchard at Traverse City, had 17 percent of its individual flower buds destroyed by a minimum temperature of -35° F. on February 9, 1934, whereas none of a considerable number of nearby trees showed a loss of more than 10 percent. On the other hand, in the spring of 1932, when these same surrounding trees had from 20 to 90 percent of their flower buds destroyed by the late type of winter-killing and by spring frost combined, selection 420 had less than 5 percent of its blossoms destroyed by these 2 types of injury together. Again, in the spring of 1934 when the killing of opened and unopened flower buds on surrounding trees ranged from 10 to 15 percent, not a single frost-injured blossom could be found on selection 420. The observation of the foreman of this orchard that for 7 consecutive years this particular tree had not failed to bear a heavy crop, while frost had levied a tax more or less frequently on surrounding trees, and that in 1928 it was 1 of only 2 trees that bore a crop when a late April freeze destroyed the crop on all other trees in the orchard, is further evidence of an exceptional degree of hardiness of its flower buds to late winter-killing and to spring frosts. Tree G in row 11 at the Graham Station orchard had none of its individual dormant flower buds destroyed by low temperatures in the winter of 1932-33 and only 9 percent in the much severer winter of 1933-34. These compare with averages of 0.7 and 11.9 for the entire orchard. Loss of individual blossoms and blossom buds from late spring frosts amounted to 79, 65, and 35 percent in 1932, 1933, and 1934, respectively, with averages for the entire orchard

⁴ ROBERTS, R. H. WINTER INJURY TO CHERRY BLOSSOM BUDS. *Amer. Soc. Hort. Sci. Proc.* 14 : 105-110, illus. 1917.

of 77, 60, and 25 percent. Obviously, the flower buds of this tree are not especially susceptible to injury from low temperatures while in the midwinter dormant state or during the short preblossoming and blossoming stages when spring frosts are likely to cause trouble. Yet in 1934 out of a random sample of 4,074 flower buds, from this tree containing an estimated 12,000 individual flowers, only 2,587 (or 22 percent) of the flowers survived the late winter type of killing. This was a far higher percentage of late winter-killing of flower buds than was found on any other tree in the orchard and indicates an especially marked susceptibility to this particular type of injury.

DISCUSSION

The data here presented on intravarietal variations in hardiness of flower buds at different stages in their development explain in part some of the irregularities in production that have been observed in Montmorency cherry orchards. A tree that is relatively productive one year and unproductive the next while the reverse is true of an adjacent tree may simply be especially sensitive or resistant to some one of the several types of injury from low temperature. The fact that the Montmorency variety, as it is ordinarily propagated and grown, evidently includes not only forms whose flower buds are relatively susceptible and relatively resistant to low temperatures at each of three fairly distinct stages but various combinations of resistance in these several stages, both indicates what to expect in the way of intravariety diversity and deterioration from a careful field survey of the losses from low temperatures and suggests that the approach to the practical solution of the problem lies in the isolation and propagation of strains whose flower buds are relatively cold-resistant at all stages of their development.

SUMMARY

Developing flower buds of the Montmorency cherry are killed by low temperatures at three different stages—while dormant, soon after growth is resumed within the bud in the spring, and in the preblossoming or blossoming stage.

Susceptibility to injury at any of these stages is not closely correlated with the degree of differentiation or development that has been attained.

The Montmorency variety includes a number of forms which show great variation in their susceptibility to low temperatures at these several stages.

Susceptibility to injury from low temperatures at one of these stages is not correlated with susceptibility at another stage.

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CORRELATION STUDIES OF EGG PRODUCTION AND POSSIBLE GENETIC INTERPRETATIONS¹

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INTRODUCTION

Many investigators have published results dealing with the correlation between each of several different variables and egg production. Their investigations have dealt with the effects, on egg production, of environmental influences, such as date of hatch and date of first egg, and with the effects of several variables that have been regarded as hereditary. It is important, from the practical as well as from the scientific viewpoint, that investigations of this kind be extended (1) to ascertain accurately the influence of the date of hatch and the date of first egg upon egg production during the first year; (2) to find adequate criteria for predicting or estimating relative egg production; and (3) if possible, to establish any linkages that may exist as indicated by the correlation coefficients. To obtain information on these points, the investigation reported in this paper was undertaken.

Several reviews of the existing literature on the subject have been published by investigators in this field, particular attention being called to those of Kempster (19, 20, 21),² Hays and Sanborn (12), and Knox (24, 25, 26).

EXPERIMENTAL PROCEDURE

In this experiment, 903 Rhode Island Red pullets and 884 Single-Comb White Leghorn pullets were used in 1928, 1929, and 1930. These birds were markedly free from disease, and only a very small percentage of the original number died during the course of the experiment. There was no selection of the pullets, and each bird that survived was kept in the laying house for 365 days from the date of first egg. All the birds were fed the same diet, housed similarly each year, and otherwise kept under as nearly the same conditions as possible in order to make the data strictly comparable. The work was carried on at the United States Animal Husbandry Experiment Station, Beltsville, Md.

The factors which were considered environmental, date of hatch and date of first egg, were studied to determine whether or not they had any influence on production and also what correlation, if any,

¹ Received for publication Jan. 9, 1935; issued June 1935.

² Reference is made by number (italic) to Literature Cited, p. 583.

they might have with possible hereditary characters. The date of hatch of the Rhode Island Reds was approximately from the middle of March to the middle of May each year, the birds being hatched in eight weekly periods. The date of hatch of the White Leghorns was from the last week in March to the middle of May, in seven weekly periods.

The possible hereditary characters considered were: Sexual maturity (age at first egg), number of eggs laid during the first 50 days after sexual maturity, number of eggs laid before March 1, percentage of production before March 1, length of winter pause, number of eggs laid during the last 50 days of the 365-day period, and number of eggs laid during August and September of the year following the date of hatch. These characters were studied in both the White Leghorns and Rhode Island Reds. One other hereditary character, broodiness, was used for Rhode Island Reds only, because practically no broodiness existed in the strain of White Leghorns. Therefore, this factor is discussed separately near the end of the paper.

Most of the foregoing characters have been studied in previous investigations. However, since some of them seem to be measures of the same hereditary characters, this study deals with the data pertaining to them in various ways in order to determine, by the use of corrected partial and multiple correlation coefficients, which of the factors may be significantly correlated with total egg production but independent of the environmental influences considered and also independent of other hereditary characters.

With respect to the partial correlation, Wallace and Snedecor (30, p. 56) state:

To the novice, a word of warning should be said concerning a phrase which has become popular in biological literature. We read of 'DE.ABCX as the partial correlation between D and E, "the other variables being held constant." As a matter of fact, 'DE.ABCX is an average of a number of simple correlations, 'DE, in groups having various values of A, B, C and D. There is no necessity of postulating the constancy of these variables. The very genius of the partial correlation coefficient is that it gives an average measure of association between two variables *independent* of the accompanying variation of the other variables. The persons using this phrase are limiting their thinking to an ideal situation in which experimental control is substituted for statistical control. This seems to us to be an unfortunately restricted attitude to take toward partial correlation.

In the interpretation of the partial correlation coefficient, it is decidedly worth while to take cognizance of this criticism by Wallace and Snedecor. All tests for the significance of simple, partial, and multiple correlations and *t* values are taken from the table for significant values for *r*, *R*, and *t* by Wallace and Snedecor (30).

RESULTS AND DISCUSSION

Table 1 contains all the simple correlation coefficients between the dependent variable, total egg production, and each of nine independent variables. Table 2 gives the means and standard deviations for the different variables. The standard deviation is useful as a measure of absolute variation in comparing the variability of one breed with that of another with respect to the same character, or of one character with another in the same breed or different breeds.

TABLE 1.—*Simple correlations between various egg-production factors and between each factor and total production in White Leghorns and Rhode Island Reds*¹

Egg-production factor	Breed	Simple correlation between factors indicated								
		Date of first egg	Sexual maturity	Eggs first 50 days	Eggs to Mar. 1	Length of winter pause	Percentage of egg production to Mar. 1	Eggs August and September	Eggs last 50 days	Total production
Date of hatch.....	White Leghorns.....	+0.605	+0.220	-0.040	-0.364	-0.119	+0.036	-0.024	-0.291	-0.158
	Rhode Island Reds.....	+0.427	+0.018	-0.031	-0.206	-0.140	+0.090	+0.078	-0.212	+0.098
Date of first egg..	White Leghorns.....		+0.901	-0.033	-0.614	-0.175	+0.033	-0.085	-0.472	-0.288
	Rhode Island Reds.....		+0.897	-0.115	-0.615	-0.209	+0.018	-0.022	-0.344	-0.240
Sexual maturity (age at first egg), days	White Leghorns.....			-0.022	-0.562	-0.155	+0.022	-0.093	-0.427	-0.272
	Rhode Island Reds.....			-0.112	-0.580	-0.171	-0.015	-0.015	-0.340	-0.296
Eggs first 50 days, number	White Leghorns.....				+0.498	-0.239	+0.619	+0.122	+0.117	+0.403
	Rhode Island Reds.....				+0.510	-0.273	+0.654	+0.134	+0.162	+0.477
Eggs to Mar. 1, number.	White Leghorns.....					-0.496	+0.727	+0.250	+0.413	+0.680
	Rhode Island Reds.....					-0.511	+0.750	+0.110	+0.342	+0.655
Length of winter pause, days.	White Leghorns.....						-0.774	-0.179	-0.054	-0.422
	Rhode Island Reds.....						-0.824	-0.125	-0.041	-0.443
Egg production to Mar. 1, percent	White Leghorns.....							+0.252	+0.172	+0.626
	Rhode Island Reds.....							+0.149	+0.150	+0.622
Eggs August and September, number.	White Leghorns.....								+0.725	+0.692
	Rhode Island Reds.....								+0.675	+0.652
Eggs last 50 days, number	White Leghorns.....									+0.660
	Rhode Island Reds.....									+0.667

¹ Correlations to be fairly significant must be 0.008 for White Leghorns and 0.062 for Rhode Island Reds; to be highly significant, they must be more than 0.094 for White Leghorns and 0.088 for Rhode Island Reds.

TABLE 2.—*Means and standard deviations for the characters studied*

Character studied	White Leghorns ¹		Rhode Island Reds ²	
	Means	Standard deviations	Means	Standard deviations
Sexual maturity (age at first egg)..... days..	180.9	27.6	199.6	29.8
Eggs first 50 days..... number ..	28.2	8.5	31.2	8.9
Eggs to Mar. 1..... do.....	77.6	26.6	74.2	28.0
Percentage of production to Mar. 1.....	53.6	14.5	56.0	16.2
Length of winter pause..... days.....	20.7	21.3	21.6	23.4
Eggs August and September..... number.....	22.3	14.8	23.6	13.3
Eggs last 50 days..... do.....	16.5	13.4	16.7	12.4
Total egg production..... do.....	191.4	46.9	191.9	44.6

¹ Mean date of hatch, Apr. 10. Mean date of first egg, Oct. 7.

² Mean date of hatch, Apr. 4. Mean date of first egg, Oct. 19.

DATE OF HATCH

It is evident from the data in table 1 that in both breeds studied there is a high and significant simple correlation between date of hatch and date of first egg. This was to be expected since it is logical to believe that the earlier a pullet is hatched the earlier she will lay her first egg.

The White Leghorn pullets have a simple correlation coefficient between date of hatch and sexual maturity of $+0.220$, which, statistically, is highly significant. The Rhode Island Reds, however, have an insignificant correlation of only $+0.018$ between these two characters. The White Leghorns also have a highly significant simple correlation of -0.158 between date of hatch and total egg production, whereas the Rhode Island Red pullets have only a barely significant coefficient of $+0.068$ between the same two characters. Therefore, the difference between the two breeds in the correlations between date of hatch and total egg production is probably due to the difference in their sexual maturity. If this assumption is true, the corrected partial correlation coefficient between the date of hatch and total egg production should be insignificant. The other variables included, were age at first egg, date of first egg, winter pause, percentage of production, and the number of eggs laid during August and September. The assumption was found to be true, the coefficients being $+0.042$ for the White Leghorns and -0.054 for the Rhode Island Reds.

Hays, Sanborn, and James (13), Kempster (21), Upp and Thompson (29), and Knox (26), obtained more or less significant and sometimes contradictory results. The differences among the data of the various investigators may be ascribed to the reasons that Kempster (21) gives in his conclusions: That the date of hatching and its association with sexual maturity is dependent on flock management, and that late hatching tends slightly to reduce the age at which the first egg is laid. In addition, it may be due to the difference in the range of the date of hatch.

Knox (26) has shown that there is a curvilinear correlation between the date of hatch and the total number of eggs produced in the pullet year for the 17 weekly hatches which he studied. Hence, different correlations might be expected, depending on the part of the curve from which the data were taken. If the data were taken from the fore part of the curve it would be positive; from the middle part, insignificant; and from the last part, negative. When the hatching period used in the present study is compared with a similar period in Knox's study, there is little evidence in either case of any significant correlation. The hatching period of 8 weeks used in this study is probably the most practical and economical one and the one that is most used by commercial poultrymen.

It is also possible that if the other investigators had used a corrected partial correlation coefficient a much lower or even an insignificant correlation would have been found between the date of hatch and total egg production.

The data of Hays (8) are at variance with the data obtained in the present study, that is, that hatching date has no significant effect upon total egg production. He states (8, p. 8):

These [coefficients of variation], together with the gross data presented in the table, show clearly that hatching date does affect annual egg production and variability in production.

It is questionable whether the data given by him show anything except normal variation. It will also be noted that as the number of individuals in each group increased materially, the coefficient of variation of the means decreased, a normal expectation. In any event, the coefficient of variation is a relative measure of variation

and shows whether or not there is more variability in one character than in another. Hence, the gross data, and not the coefficients of variation, presented by him showed the effect of hatching date upon annual egg production.

If hatching date affects egg production, the gross data of Hays (8) for the 4 years, if compiled in eight weekly hatches, should show a definite trend in egg production. When the data are thus compiled, it is seen that hatching date has little or no effect on egg production. What trend is apparent is directly opposite to that usually reported, which is that the earlier a bird is hatched the more eggs she will lay in her first laying year. However, when the average egg production of the birds from the first 4 hatches is compared with the average egg production of the birds from the last 4 hatches it is found that there is a difference of only 16+ eggs, hardly enough to be significant. Even if the difference were significant it would still be necessary to find out whether or not there would be a significant partial correlation. As has been shown in this investigation, it is possible to obtain a significant correlation coefficient and yet have an insignificant corrected partial correlation coefficient.

DATE OF FIRST EGG

The correlation coefficients of +0.901 and +0.897 for the White Leghorn and Rhode Island Red pullets, respectively, between date of first egg and sexual maturity are very high (table 1). Sexual maturity is obtained by determining the number of days elapsing between the date of hatch and the date of first egg. Hence, if the hatching date were held constant, sexual maturity would be the controlling factor in establishing the date of first egg, in which case the simple correlation coefficient between sexual maturity and date of first egg would be 1.00. Even when the date of hatch varies over an interval of 7 or 8 weeks, as was the case in the present study, a high correlation may be expected between these two variables. The high correlation coefficients between them in this study agree with the data of Jull (16), and with the data of Knox (25) when the date of hatch was limited to a period of 7 or 8 weeks.

The simple correlation coefficients between date of first egg and total egg production were found to be highly significant in both breeds, -0.288 for the White Leghorns and -0.240 for the Rhode Island Reds. However, as stated by Knox (24, p. 249)—

It would be of considerable value for predicting the future egg production of pullets to know exactly what this association would mean statistically and whether or not the date of first egg is more important than sexual maturity.

He further states (24, p. 249):

* * * it is difficult to differentiate between them, but a multiple correlation study and the use of partial regression coefficients ought to give a true indication of the actual statistical value of each with winter, spring, and annual egg production.

With this in mind, the writers calculated the partial correlation coefficient between the date of first egg and total egg production and found it to be of insignificant value in both breeds, being -0.016 for the White Leghorn and -0.055 for the Rhode Island Red pullets. In obtaining the partial correlation coefficients, the other variables included were date of hatch, sexual maturity, percentage of produc-

tion to March 1, and egg production during August and September of the year following date of hatch.

The data presented concerning the simple correlation coefficients agree with the data of Jull (16), Kempster (20), Maw and Maw (27), Hauschildt (7), and Knox (25). However, Kempster and Knox each found a curvilinear relationship between date of first egg and total egg production, which was probably due to the fact that their data included a greater range in the date of hatch and date of first egg than is included in this investigation. When the data of the different investigators are compared empirically month for month the same relative association is found as for the period considered in this investigation. However, the partial correlation coefficient, which none of the previous investigators included, shows that the date of first egg is not significantly correlated with total egg production and that the significant simple correlation coefficients found in this study, and probably in the previous investigations mentioned, are due to some other factor, possibly sexual maturity.

Therefore, within the limits of this study, neither of the two environmental factors considered—date of hatch and date of first egg—has any significant correlation with total egg production, as shown by the corrected partial correlation coefficient with total egg production.

SEXUAL MATURITY

Sexual maturity, already defined as the number of days that elapse between the date that the pullet is hatched and the date that she lays her first egg, is the first hereditary character considered in this paper. The same character has been variously designated by other investigators as age in days laying commences, maturity, sexual maturity, and age at first egg.

The simple correlation coefficients, between sexual maturity and total egg production, of -0.272 for the White Leghorns and -0.296 for the Rhode Island Reds are relatively large and highly significant. The partial correlation coefficients are even higher, -0.398 for the White Leghorns and -0.479 for the Rhode Island Reds, the other variables considered being winter pause, percentage of production to March 1, and number of eggs during August and September of the year following the date of hatch. Hence it is apparent that sexual maturity is highly and significantly correlated with total egg production.

Similar observations concerning the simple correlations have been made by Ball and Alder (1), Kennard (22), Hervey (14), Hays and Bennett (9), Hays, Sanborn, and James (13), Jull (16, 17), Kempster (19, 20), Parkhurst (28), Knox (23), Buster (2, 3), and Graham.³ None of these investigators calculated any partial correlation coefficients.

The simple correlation coefficients between sexual maturity and total egg production obtained by Hays and Sanborn and by the present authors are in agreement, but the partial correlation coefficients are not. Hays and Sanborn obtained a partial correlation coefficient of -0.0238 ± 0.0177 , which is insignificant. The authors, on the other hand, obtained a significant partial correlation co-

³ GRAHAM, J. C. THE CORRELATION OF BODY WEIGHT AT FIRST EGG AND AVERAGE MONTHLY AND ANNUAL EGG WEIGHT IN RHODE ISLAND REDS. (Unpublished manuscript.)

efficient of -0.479 for the same breed. This seeming lack of agreement may be due, however, to the highly significant simple correlation coefficient, -0.5956 , which Hays and Sanborn observed between sexual maturity and their particular measure of persistence. Their measure of persistence, which is discussed more fully later, seems questionable because the high correlation between sexual maturity and the measure of persistence that they used was probably responsible for lowering the partial correlation coefficient between sexual maturity and egg production.

RATE OF LAYING

Four different measures of the rate of laying were studied, namely: Number of eggs laid during the first 50 days of production, number of eggs laid to March 1, length of winter pause, and percentage of production to March 1. There may be some question as to whether these four measures of rate are comparable. If they are comparable, then a high correlation coefficient should be obtained between each pair of the four measures and similar correlations should be obtained between each measure and total egg production.

The correlation coefficient between the number of eggs laid the first 50 days and date of hatch is insignificant for both the White Leghorns and the Rhode Island Reds (table 1). The correlations between the former character and each of the two characters, date of first egg and sexual maturity, although insignificant in the case of the White Leghorns, are low but highly significant in the case of the Rhode Island Reds. The correlations between the number of eggs the first 50 days and each of the four characters, eggs to March 1, winter pause, percentage of production to March 1, and total production, are high and significant in both breeds.

Evidently the number of eggs laid the first 50 days is influenced relatively little by the date of hatch and has practically no correlation with date of first egg and sexual maturity, except in the Rhode Island Reds, in which case the correlation coefficient is -0.115 . However, it has a high and significant simple correlation with the other measures of rate and with egg production. The number of eggs laid the first 50 days has a higher partial correlation with egg production than the simple correlation coefficient, being $+0.461$ and $+0.518$ for the White Leghorns and Rhode Island Reds, respectively. This measure of rate, therefore, is a good one to use, provided no other one has a higher partial correlation coefficient with annual egg production and no significant correlation coefficient with environmental factors or other hereditary factors.

The number of eggs laid to March 1 has been considered by several investigators as winter egg production. Table 1 shows that the number of eggs laid to March 1 is highly and significantly correlated with the date of hatch and the date of first egg in both breeds. It is also highly correlated with the other measures of rate and with total egg production. The partial correlation between the number of eggs to March 1 and total egg production was found to be $+0.605$ for the White Leghorns and $+0.735$ for the Rhode Island Reds.

Because this measure of rate is highly correlated with the two environmental factors studied, it is not so desirable a measure to use as is the number of eggs laid in the first 50 days. This seems to be

obvious even though the number of eggs laid to March 1 has a higher correlation with the other measures of rate and with egg production.

The number of eggs laid before March 1 has been used as a measure of rate by many investigators in genetic and statistical studies of egg production. When the previously mentioned facts regarding this factor are considered, it is evident that any adequate genetic or statistical interpretation is not only difficult but hazardous and in all probability should not be used in a study of egg production. This, of course, does not preclude the importance of economic studies of winter egg production.

Winter pause, another measure of rate studied, has been considered variously by different investigators. In this investigation, winter pause is considered as the accumulated number of days that production has ceased for periods of 4 days or more.

The data in table 1 show that winter pause, as defined, is significantly correlated with the date of hatch, date of first egg, and sexual maturity. Any one of these correlations would be sufficient to eliminate it from consideration, even though it is highly correlated with the other measures of rate and with total egg production. This is substantiated by the partial correlation coefficients of $+0.049$ for the White Leghorns and -0.077 for the Rhode Island Reds between winter pause and total egg production, when percentage of production to March 1 is used as one of the other variables. These coefficients show that winter pause has very little actual correlation with total egg production. Therefore, such a measure is not only significantly correlated with the environmental factors studied but as a reciprocal is more or less a statistical duplication of the percentage of production to March 1.

In addition to this, winter pause is difficult to use in studies on egg production. In this connection, Goodale and Sanborn (5, p. 115) made the following observation:

The trait [winter pause] does not lend itself to ordinary statistical treatment because of its nature, which depends partly on an inherent condition of the strain, and partly on environmental conditions, particularly those that determine the time of year when birds begin to lay. Further, it is possible that more than one cycle is involved.

Then, again, Jull (18, p. 226, 227), in his study of factors affecting egg production, states that—

No attempt has been made to separate the pauses occurring in each bird's record according to whether they were caused by external factors or were due to inheritance, because no method has been made known whereby the facts can be determined

Therefore, because of the difficulty of determining a satisfactory measure of winter pause, the inability to separate the influence of environment and inheritance, its doubtful value as shown by the present correlation study, and its insignificant partial correlation with egg production, winter pause should be eliminated from consideration in any attempt to solve the problem of the inheritance of egg production.

The last measure of rate used is the percentage of egg production from the date of first egg to March 1. This measure has been termed "intensity of production" by some writers. Hurst (15) was probably the first investigator to study this particular measure of rate. Few

investigators have seen fit to use it, yet the data in table 1 show that it is ideal. It has practically no correlation with the date of hatch for the White Leghorns, and a coefficient of only $+0.090$ for the Rhode Island Reds and practically no correlation with date of first egg and sexual maturity for either breed. In addition, it has a high simple correlation coefficient with total egg production ($+0.626$ for the White Leghorns and $+0.622$ for the Rhode Island Reds) and a correspondingly high partial correlation coefficient ($+0.555$ for the White Leghorns and $+0.568$ for the Rhode Island Reds).

The data agree with those of Hays and Sanborn (11), who obtained a correlation coefficient of $+0.48 \pm 0.01$ between rate of production (percentage of production to March 1) and total annual production for 2,528 Rhode Island Red pullets. Knox (23) obtained a correlation coefficient of $+0.34 \pm 0.08$ between rate of production based on the 2 consecutive months of highest production (another measure of rate) and total annual production in White Plymouth Rocks.

However, the data of Hays and Sanborn (11) showed a significant correlation in Rhode Island Reds between rate of production and sexual maturity, the coefficient being -0.23 ± 0.01 , whereas the present authors found a correlation coefficient of $+0.022$ in the White Leghorns and of -0.015 in the Rhode Island Red pullets, respectively, signifying practically no correlation. It may be that the lack of agreement is due to the different methods of calculating rates. Hays and Sanborn (11) calculated rate, as reported by them (10), by using the number of eggs laid from the date of first egg to March 1, divided by the number of days from first egg to March 1, less all pauses of 4 or more days from November 1 to March 1. The authors figured percentage of production to March 1 in a similar manner but included the pauses, a method which seems less arbitrary than the one used by Hays and Sanborn (11).

From these data it is apparent that percentage of egg production from date of first egg to March 1 should be used to a greater extent in future statistical and genetic investigations of egg production than has been done in the past. It is much to be preferred to the more generally used measure of rate, the number of eggs to March 1, or to winter pause. It is also a better measure of rate than the number of eggs laid in the first 50 days of production as measured by the size of the correlation coefficients with egg production and lack of correlation with the environmental factors used.

PERSISTENCE

The two measures of persistence chosen were the number of eggs laid during the last 50 days of the 365-day period and the number of eggs laid during August and September of the year following the date of hatch of the pullets. These were studied in the same manner as the different measures of rate.

As shown by table 1, the number of eggs laid during the last 50 days of the laying year is evidently highly and significantly correlated with the environmental factors considered and with sexual maturity and percentage of production to March 1, although least with respect to the latter. It also has a very high and significant simple correlation coefficient with total egg production, being $+0.660$ and $+0.667$ for the White Leghorns and Rhode Island Reds, respectively. The

partial correlation coefficients were slightly higher in both breeds, being +0.667 for the White Leghorns and +0.700 for the Rhode Island Reds. In spite of these high correlations with total egg production, the number of eggs laid the last 50 days should be eliminated from all statistical and genetic studies of egg production because of the high correlations between this factor and each of the following: Date of hatch, date of first egg, sexual maturity, and percentage of production before March 1.

The simple correlation coefficients of the number of eggs laid in the last 50 days with date of hatch, sexual maturity, percentage of production to March 1, and total egg production, agree substantially with the results reported by Hays and Sanborn (10, 12). The same is true of the partial correlation coefficients. This would indicate that the number of eggs laid the last 50 days of the 365-day period, used by the authors, and the length of the laying period tabulated in class intervals of 15 days, the method used by Hays and Sanborn (10), are similar measures of persistence. Harris and Lewis (6) obtained a correlation coefficient of -0.2438 ± 0.0302 between date of first egg and date of cessation of laying, which agrees substantially with the correlation coefficient given in table 1 between date of first egg and number of eggs laid the last 50 days of the laying year. The reasons given by the authors for eliminating from consideration the number of eggs laid in the last 50 days of the laying year are no doubt applicable to the length of the laying period, and probably to the date of cessation of laying as reported by Hays and Sanborn (10) and Harris and Lewis (6), respectively.

The other measure of persistence, the number of eggs laid during August and September of the year following the date of hatch, was found to be a better measure of persistence than the number of eggs laid during the last 50 days of the laying year, as shown by the data in table 1.

There are evidently no highly significant correlations in either breed between the number of eggs laid during August and September and the date of hatch, date of first egg, and sexual maturity. This measure of persistence is highly and significantly correlated with percentage of production to March 1 in both breeds, the simple correlation coefficients being +0.252 and +0.149 and the partial correlations +0.078 and +0.164 for the White Leghorns and the Rhode Island Reds, respectively. This may be due to the linkage of some of the major genes for egg production, but it is more likely to be due to the fact that percentage of production is calculated from a part of the whole (egg production), and persistence is measured as a part of the same whole, and both, in a way, measure the same trait at different periods. The number of eggs laid during August and September may be considered as an expression of rate late in the laying year, as well as persistence. For instance, a bird that lays 30 eggs during this period would have a late rate of approximately 50 percent. It would be natural, therefore, to expect some correlation between number of eggs laid during August and September and percentage of production to March 1. As a matter of fact, it is surprising that the correlation coefficient between them is so low. Furthermore, the partial correlation coefficient between persistence and egg production, which includes percentage of production to March 1 as one of the

variables, is higher than the simple correlation coefficient, being $+0.733$ for the White Leghorns and $+0.772$ for the Rhode Island Reds. This would indicate that even when the percentage of production to March 1 is used as one of the variables there was no lowering of the correlation between the number of eggs laid during August and September and total egg production.

The data in table 3 show the correlation coefficients between persistence and various factors, obtained by Hays and Sanborn (11) and the present authors. Since the simple correlation coefficients between the number of eggs the last 50 days and Hays and Sanborn's measure of persistence are not significantly different, the two measures may be considered the same. These correlations were obtained even though the Rhode Island Red population (signifying both number of birds and various strains) was different in each case and different years were involved. The data in this table show also that the number of eggs laid during August and September is the only measure of persistence that lacks a significant correlation with sexual maturity, and it has the lowest correlation with the date of hatch and with sexual maturity. Hence, the number of eggs laid during August and September evidently is the best measure of persistence.

TABLE 3.—*Comparison of correlation coefficients between different measures of persistence and the factors indicated, for Rhode Island Reds*

Item of comparison	Investigators and measure of persistence used		
	Hays and Sanborn ¹	Knox, Jull, and Quinn	
		Last 50 days	August and September
Simple correlation coefficient between persistence and—			
Total egg production.....	+0.708	+0.667	+0.652
Percentage of production to Mar. 1.....	+ .184	+ .150	+ .149
Sexual maturity.....	-.615	- .340	-.015
Date of hatch.....	-.221	- .212	+ .078

¹ Their measure of persistence was a class interval of 15 days with a range of from 67 to 366 days.

The evidence presented does not necessarily show that linkage exists between sexual maturity and persistence, for the relation between the two might be due to an inadequate measure of persistence. This line of reasoning would invalidate the use of the low partial correlation coefficients reported by Hays and Sanborn (11) between sexual maturity and total production because of the high simple correlation between sexual maturity and the measure of persistence used.

MULTIPLE CORRELATIONS

In considering the problem from the standpoint of a multiple correlation study, the authors formulated table 4. The method of presentation is predicated, in part, on the deductions of Ezekiel (4, p. 261), who states:

* * * it is evident that the reliability of a regression coefficient varies directly with the multiple correlation of the dependent factor with the other factors, but inversely with the multiple correlation of the particular independent

factor with the other independents. The more closely a particular independent factor can be estimated from the other factors present, the less accurately can the net relation of the dependent factor to it be determined.

In calculating R and \bar{R} in table 4, the authors used total egg production as the dependent variable with six independent variables. R represents the multiple correlation coefficient, and multiple \bar{R} the most probable correlation for the fowls of the universe. Date of hatch, date of first egg, sexual maturity, and winter pause were used in each of the correlations. The other two independent variables consisted of some measure of rate and persistence, as shown in table 4. Various measures of rate and persistence were substituted for others to determine whether the different measures would improve the prediction value.

TABLE 4.—Multiple correlations of six variables with total egg production

Four variables ¹ and—	White Leghorns		Rhode Island Reds	
	R	\bar{R}	R	\bar{R}
Number of eggs August and September; percentage of production to Mar. 1	0.874	0.873	0.887	0.886
Number of eggs August and September; number of eggs to Mar. 1	.876	.875	.894	.893
Number of eggs August and September; number of eggs first 50 days	.846	.844	.865	.864
Number of eggs last 50 days; percentage of production to Mar. 1	.841	.840	.855	.854

¹ Date of hatch, date of first egg, sexual maturity, and winter pause.

The table shows that there is little difference between the multiple R and \bar{R} for the different measures substituted. In only one case was the difference significant. When the number of eggs laid in August and September was substituted for the number laid during the last 50 days, the \bar{R} was increased from 0.840 to 0.873 in the case of the Leghorns and from 0.854 to 0.886 in the case of the Rhode Island Reds. As the value of p is between 0.01 and 0.02, the difference between the \bar{R} 's was significant in both breeds. The number of eggs laid in August and September, therefore, is the more valuable measure of persistence.

Since there is no material difference in the multiple correlation coefficients when different measures of rate are used, as shown in table 4, it is evident that they are measures of the same trait. Hence, from the discussion of the various simple, corrected partial, and multiple correlation coefficients, the following statements may be made: It is evident that when the date of hatch and the date of first egg occur at the proper time and within the limits of this experiment, they have no significant effect upon total egg production. Furthermore, the best measures of rate and persistence to use are the percentage of production before March 1 and the number of eggs laid during August and September of the year following the date of hatch. These two measures may be considered as hereditary characteristics, as is sexual maturity. From the foregoing evidence it would be expected that a multiple correlation coefficient between total egg production and the three hereditary characteristics just mentioned would be practically of the same magnitude as the highest of those shown in table 4, which include six independent variables.

Therefore, the multiple correlation coefficients between total egg production and the three major factors, sexual maturity, percentage of production to March 1, and eggs laid during August and September, were calculated. For the White Leghorns the R and \bar{R} were each found to be 0.866; for the Rhode Island Reds, 0.887 and 0.886, respectively. For each breed the highest multiple correlation coefficient (R) is not materially different from the highest one given in table 4. The multiple correlation coefficient for the universe (\bar{R}) in the Rhode Island Reds, 0.886, is the same as for the same variables used in table 4, which included, in addition, the variables date of hatch, date of first egg, and winter pause. This correlation (\bar{R}) for the White Leghorn pullets is not significantly different. It is evident, therefore, that there is little to be gained by the addition of any more independent variables studied than the three major ones previously mentioned.

That these major variables add significant amounts to correlation is shown by the following: For the White Leghorns and Rhode Island Reds the simple correlation between number of eggs laid during August and September and total egg production is 0.692 and 0.652, respectively. When percentage of production to March 1 is added in a multiple correlation study, the multiple correlation coefficient is 0.839 and 0.850, respectively. The further addition of sexual maturity increases the coefficient to 0.866 and 0.886, respectively.

BROODINESS

Correlations were obtained between broodiness in Rhode Island Reds and various egg-production factors. The simple correlation with sexual maturity was +0.208; with percentage of production to March 1, -0.148; with number of eggs laid during August and September of the year following date of hatch, -0.156; and with total production, -0.342. Between broodiness and total production the corrected partial correlation coefficient was -0.260, R was 0.894, and \bar{R} was 0.894, when sexual maturity, percentage of production to March 1, and the number of eggs laid during August and September were considered as additional variables. Because there was little broodiness in the White Leghorns, their records were not used in the correlation coefficient studies.

All the correlation coefficients are significant. They apparently indicate that later maturing birds are more likely to be broody than early maturing birds and that broodiness tends to reduce the rate and persistence of production. It may be that the correlation between broodiness and sexual maturity indicates some linkage between them. In any event, it would tend to complicate the interpretation of the inheritance of broodiness. Although the multiple correlation (0.894) is higher than the correlation (0.887) between total egg production and the three major factors previously mentioned, the difference is not significant. This may be due to selection over a period of years against broodiness, which would tend to lower the correlation.

The data of Hays and Sanborn (12) agree with the data of the authors with the exception of the low correlation which they obtained between broodiness and their particular measure of persistence. The differences between the findings of the authors and of Hays and Sanborn may be due to different populations and selection of nonbroody birds over a long period of time.

Hays and Sanborn (12) obtained a multiple correlation of 0.864 between egg production in Rhode Island Red pullets and five traits, but they used different measures of rate and persistence than were used in this study. The present authors obtained a multiple correlation of 0.894 for the Rhode Island Red data when four independent variables were used, and 0.887 when only three variables were used. The difference between the two coefficients 0.894 and 0.864 is highly significant, since the value of t is greater than 3. Even if the difference were insignificant, it would indicate that the addition of other measures, which are more or less duplicates, add practically nothing to the multiple correlation.

SUMMARY AND CONCLUSIONS

The records of 903 Rhode Island Red pullets and 884 Single-Comb White Leghorn pullets were used in 1928, 1929, and 1930 to ascertain the influence of the date of hatch and the date of first egg upon egg production during the first year, to find adequate criteria for predicting or estimating relative egg production, and to establish any linkages that might exist as indicated by the correlation coefficients.

It was found that the date of hatch and date of first egg have an insignificant effect upon egg production when the date of hatch is during the normal hatching season and of 7 or 8 weeks' duration, the period covered by this study. This fact was ascertained through the use of partial correlation coefficients. Between date of hatch and egg production these coefficients were +0.042 for the White Leghorns and -0.054 for the Rhode Island Reds, and between date of first egg and egg production they were -0.016 for the White Leghorns and -0.055 for the Rhode Island Reds.

The following known measures of hereditary traits that control egg production were used in this study: Sexual maturity (age at first egg), number of eggs laid the first 50 days, number of eggs laid to March 1, length of winter pause, percentage of production to March 1, number of eggs laid during August and September of the year following date of hatch, number of eggs laid the last 50 days of the first laying year, and broodiness.

Between sexual maturity and total egg production, the partial correlation coefficients, which were -0.398 for the White Leghorns and -0.479 for the Rhode Island Reds, were highly significant even though percentage of production to March 1, length of winter pause, and the number of eggs laid during August and September were included as the other variables.

It was found that the number of eggs laid the first 50 days, the number of eggs to March 1, length of winter pause, and percentage of production to March 1 were all measures of the same trait, rate of production. The best of these measures was found to be the percentage of production to March 1. This measure of rate had the smallest correlation coefficient with each of the environmental factors studied and, with the exception of the number of eggs the first 50 days, with sexual maturity also. In addition, the percentage of production to March 1 had a high partial correlation coefficient with egg production, +0.555 and +0.568 for the White Leghorns and Rhode Island Reds, respectively.

The number of eggs laid during August and September of the year following the date of hatch and the number of eggs laid the last 50

days of the first laying year were found to be similar measures of persistence of production. The better of these two measures was the number of eggs laid during August and September. The partial correlation coefficients between this measure of persistence and egg production were +0.733 for the White Leghorns and +0.772 for the Rhode Island Reds, these coefficients being higher than those between the other measure of persistence and egg production. The number of eggs laid during August and September had a much smaller correlation coefficient with each of the environmental and hereditary factors considered, except length of winter pause and percentage of production to March 1, than the number of eggs laid the last 50 days.

Broodiness was studied in the Rhode Island Red pullets only, as very little broodiness was encountered in the White Leghorns that were used. A partial correlation coefficient of -0.260 was obtained between broodiness and egg production. It was found also that the broody hens, on an average, matured later, laid at a slower rate, and were less persistent in production than the nonbroody hens.

The three major hereditary traits—sexual maturity, rate as measured by percentage of production to March 1, and persistence as measured by number of eggs laid during August and September—had a high multiple correlation with first-year egg production in both breeds. The multiple correlation coefficients, R , were 0.866 and 0.886 for the White Leghorns and Rhode Island Reds, respectively. When the fourth trait, broodiness, was added to these major traits in a multiple correlation study in the Rhode Island Reds, the coefficient, R , was found to be 0.894. Other factors that were studied, when added to the three major traits mentioned, produced no significant change in the size of the multiple correlation coefficient.

No evidence of linkage was found in either breed between the best measure of rate—percentage of production to March 1—and sexual maturity, as indicated by the coefficients. The simple correlation coefficients were very insignificant, being +0.022 and -0.015 for the White Leghorns and Rhode Island Reds, respectively. The simple correlation coefficients between the better measure of persistence—the number of eggs laid during August and September—and sexual maturity were -0.093 and -0.015 for the White Leghorns and Rhode Island Reds, respectively, indicating that there was comparatively little significance or linkage between these factors.

Partial correlation coefficients of +0.078 in the case of the White Leghorns and +0.164 in the Rhode Island Reds were found between percentage of production to March 1 and the number of eggs laid during August and September. These significant correlations are probably due to the fact that these two traits are measures of different parts of the whole (first-year egg production) and in a way are measures of the same thing at different periods, rather than being due to any linkage relationship. Even though they are calculated from different parts of the whole, the partial correlation coefficients are low enough to permit the use of both rate and persistence as criteria of egg production.

The simple correlation coefficients between the number of eggs laid during August and September and first-year egg production were +0.692 for the White Leghorns and +0.652 for the Rhode Island Reds. When the multiple correlation coefficients were obtained between the number of eggs laid during August and September, the

percentage of production to March 1, and total egg production, the last-mentioned factor being used as the dependent variable, the coefficients obtained were 0.839 and 0.850 for the White Leghorns and Rhode Island Reds, respectively. The differences between the simple and multiple correlation coefficients were 0.147 and 0.198, and are highly significant. Therefore, as the percentage of production to March 1 adds a significant amount to the simple correlation coefficient between the number of eggs laid during August and September and egg production, it is desirable that both percentage of production to March 1 and the number of eggs laid during August and September be included in statistical and genetic investigations of egg production.

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CYTOGENETICS OF TETRAPLOID MAIZE¹

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INTRODUCTION

Tetraploidy is unknown in normal stocks of maize, although large numbers of individuals have been examined cytologically and inspected under field conditions for the purpose of detecting tetraploids. In other plant genera, such as *Oenothera*, *Datura*, and *Primula*, which have been used extensively in breeding experiments, tetraploid individuals have been discovered at infrequent intervals. These tetraploid mutants with twice as many chromosomes as the normal diploids ordinarily exhibit gigas, or giant characteristics. Plant species with chromosome numbers in multiples of some base number such as 5, 7, 9, etc., are of wide-spread occurrence in nature. This fact suggests that chromosome doubling has played an important part in the evolution of new species.

The experimental production of tetraploid maize (*Zea mays* L.) was accomplished by subjecting plants in the early embryonic stage to high temperatures for short periods.² The treatments of the young ears were carefully timed to coincide with the first cell divisions in the development of the proembryo from the zygote. This was done in order to obtain individuals that would be entirely tetraploid.³ Tetraploids were induced by the heat treatments with a frequency ranging from 2 to 5 percent. In addition, aborted, scarred, and defective grains and grains with mosaic endosperms were produced on the treated ears (fig. 1).

APPEARANCE OF TETRAPLOIDS

The giant characteristics typical of most tetraploids are only moderately developed in the vegetative organs of maize tetraploids. But the structures associated with the process of reproduction are definitely enlarged. In general appearance maize tetraploids do not differ markedly from the related diploid plants (fig. 2). They are of about the same height and have a similar habit of growth. However, their stalks are thicker and sturdier and the leaves are somewhat broader and thicker. The tassels also are larger and the individual parts of the staminate inflorescence are conspicuously enlarged. Abundant

¹ Received for publication Nov. 27, 1934; issued June 1935. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Botany, New York State College of Agriculture, Cornell University. This investigation was aided by a grant from the committee on effects of radiation upon living organisms, of the Division of Biology and Agriculture, National Research Council.

² RANDOLPH, L. F. SOME EFFECTS OF HIGH TEMPERATURE ON POLYPLOIDY AND OTHER VARIATIONS IN MAIZE. Natl. Acad. Sci. Proc. 18: 222-229. 1932.

³ RHOADES, V. H. A STUDY OF FERTILIZATION IN *ZEa MAYS*. (Master's thesis, Cornell Univ.) 1934. [In manuscript.] Rhoades determined the time interval from pollination to fertilization in maize at a constant temperature of 25° C. With silk lengths of 7 to 11 cm the interval was 15 hours; with silks 11 to 14 cm long the interval was 17 hours. The primary endosperm nucleus was found to divide almost immediately after fertilization, and 4 to 8 endosperm nuclei were present at the time of the division of the zygote 10 to 12 hours after fertilization.

pollen of uniform size but much larger than that of diploids is produced by the $4n$ plants (fig. 3, *A, B*). From the seedling stage to maturity the $4n$, as compared with the $2n$ individuals of the same stage in development, have larger and more widely spaced stomata and correspondingly larger epidermal cells (fig. 3, *C, D*). These differences serve as useful criteria for identifying the tetraploids.

The ears and kernels of tetraploid maize are distinctly larger than those of comparable diploid stocks. This fact was clearly demonstrated by an experiment in which tetraploidy was induced in the F_1 hybrid

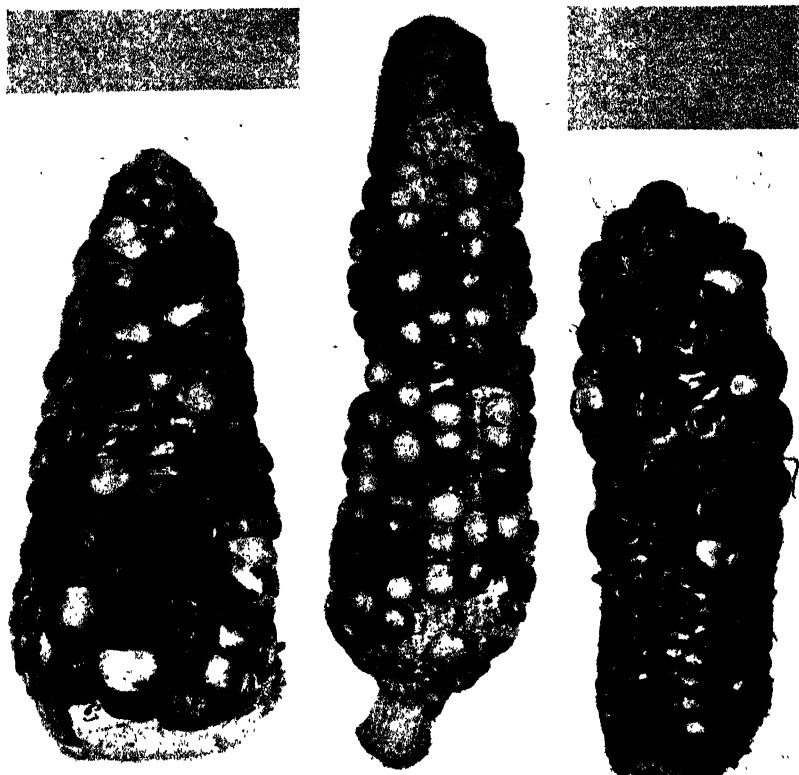


FIGURE 1.—Mature ears of maize treated with heat shortly after fertilization to induce tetraploidy. In addition to the production of tetraploids, the normal frequency of defective, scarred, and mosaic grains also was markedly increased by the heat treatments.

between two similar inbred lines and in which a comparison was made between self-pollinated tetraploid and diploid lines derived from the induced tetraploids and their diploid sibs. In figure 4 are illustrated ears that are representative of the parental diploid lines, the F_1 diploid hybrid, and a derived tetraploid line.

To obtain an indication of the relative weights of the $2n$ and $4n$ kernels in these lines, several samples of shelled grain, each containing 1,000 kernels, were weighed. The average weight of three such diploid samples was 221.6 g; the average for the same number of tetraploid samples was 330.2 g, or approximately 50 percent more. However, the $4n$ ears were not so well filled as those of the diploid,

and consequently the kernels were less crowded on the ear, a condition which probably accounted for part of their increased weight. In figure 5 are shown samples of the shelled grain from the lines used in making the weight comparisons.



FIGURE 2 - Normal diploid (A) and induced tetraploid (B) sister maize plants from the progeny of heat-treated ears.

BREEDING BEHAVIOR

When selfed or intercrossed, maize tetraploids produce large ears with large, well-filled kernels. Partially developed or defective kernels are rarely formed. Pollen is shed freely, and ordinarily $4n$ plants produce no more shriveled or aborted pollen than the 3 to 5 percent normally produced by diploid stocks. The fertility of the

tetraploids is somewhat reduced, as indicated by the presence of aborted ovules and an irregular spacing of the kernels on the mature ears. In comparison with diploid stocks the amount of reduced fertility varied from 5 to 20 percent in the $4n$ stocks thus far produced.

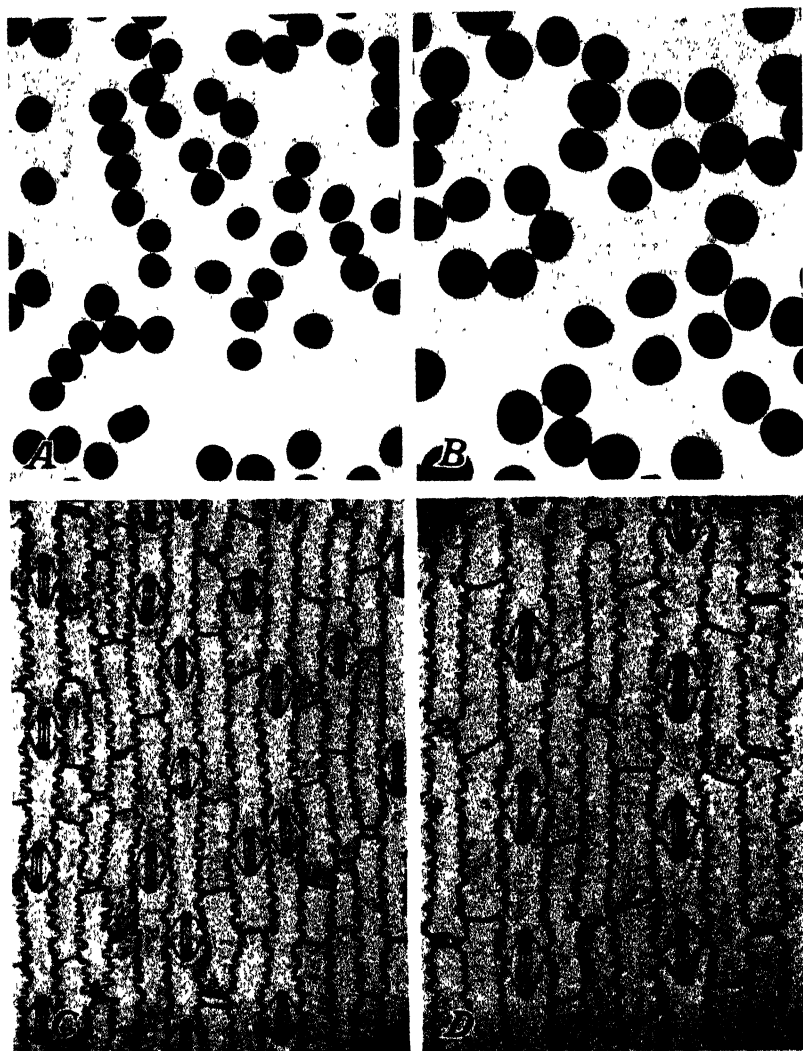


FIGURE 3.—Photomicrographs of the pollen of diploid (A) and tetraploid (B) maize, and of the lower epidermis of the leaves of mature diploid (C) and tetraploid (D) maize. $\times 55$.

In exceptional cases a higher percentage was found in individuals which also had some aborted pollen. This type of infertility apparently is not due to the doubling of the chromosomes, since it occurs in both the diploid and tetraploid progenies of heat-treated plants.

Tetraploid stocks breed true for tetraploidy with only slight deviations in chromosome number in subsequent generations. Mater-

nal diploids, which are to be expected at infrequent intervals in the progeny of tetraploids, provided parthenogenesis occurs in tetraploids as in diploids, thus far have not been observed.

Since tetraploids have 4 sets instead of 2 sets of homologous chromosomes, the Mendelian ratios obtained from them are very different from those obtained from diploids.⁴ The selfed ears from

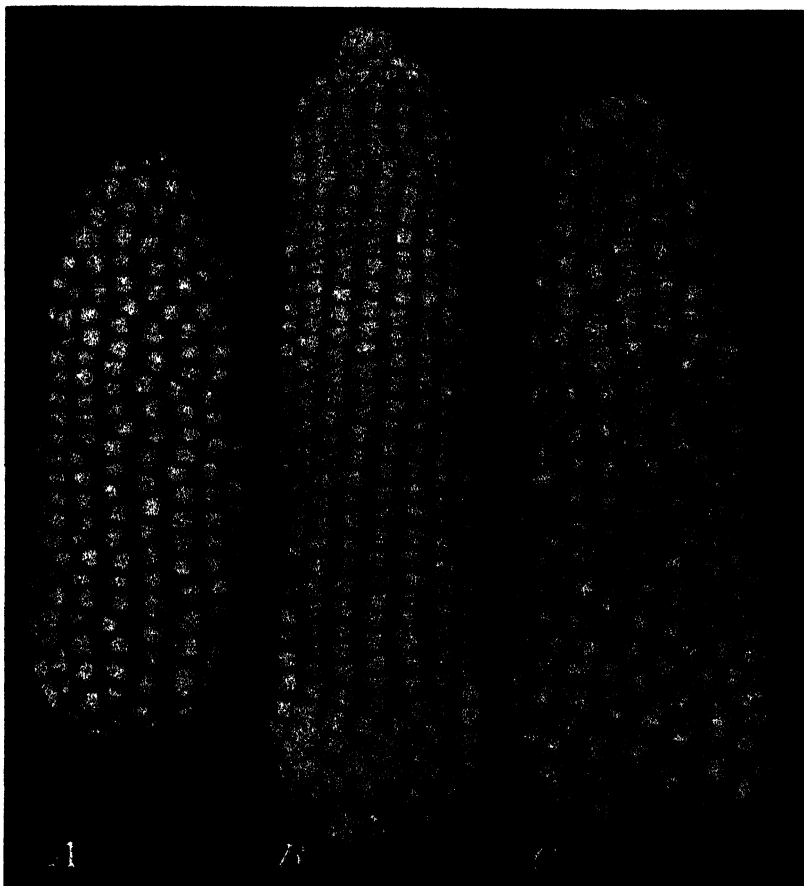


FIGURE 4.—Representative ears of an inbred line (A), the F_1 hybrid (B) between line A and a similar line, and a tetraploid line (C) derived from the F_1 hybrid and inbred for two generations.

$2n$ and $4n$ plants shown in figure 6 illustrate typical diploid and tetraploid F_2 segregations for the aleurone factor pair Rr . They approximate the expected 3:1 and 35:1 ratios. Similar segregations for other seed, seedling, and mature plant characters also have been noted in tetraploid maize stocks, but the data are inadequate to determine whether or not there are significant deviations from the expected ratios.

⁴ BLAKESLEE, A. F., BELLING, J., and FARNHAM, M. E. INHERITANCE IN TETRAPLOID DATURAS. *Bot. Gaz.* 76: 329-373. 1923.

MÜLLER, H. J. A NEW MODE OF SEGREGATION IN GREGORY'S TETRAPLOID PRIMULAS. *Amer. Nat.* 48: 508-512. 1914.

TETRAPLOID AND DIPLOID INTERCROSSES

The intercrossees between diploid and tetraploid maize exhibited a high degree of sterility in both direct and reciprocal crosses. The resulting progeny with rare exceptions were triploid hybrids. When the diploid was the seed parent the fertility, as measured by the proportion of the viable to the aborted and defective seed, was less than 0.5 percent. When the tetraploid was the seed parent there was approximately 5 percent fertility. Mixtures of approximately equal amounts of pollen from $4n$ and $2n$ stocks applied to the silks of the $4n$ and $2n$ parent plants resulted in a set of seed by the diploid almost exclusively from its own pollen; the tetraploid, however, set very little seed from its own pollen, the majority being defective kernels from the pollen of the diploid. The pollen of the tetraploid apparently

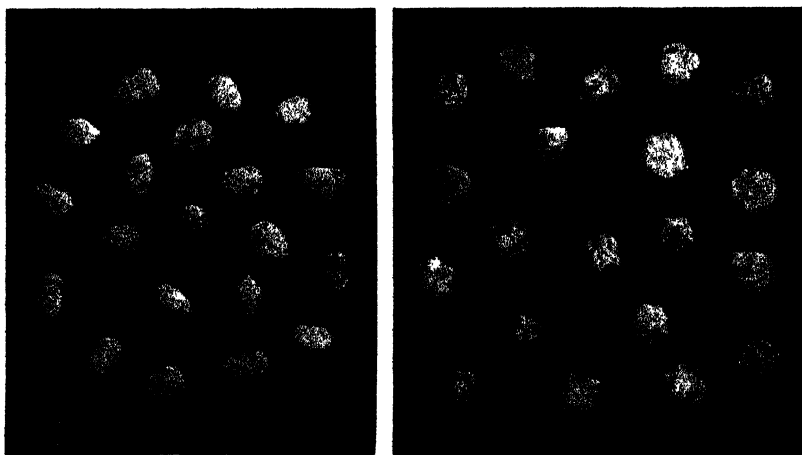


FIGURE 5.—Diploid (A) and tetraploid (B) kernels of maize from 2-generation inbred $2n$ and $4n$ lines derived from an F_1 hybrid between inbred strains with kernels of a similar type.

was unable to compete favorably with that of the diploid either on its own silks or on those of the diploid.

The crossing behavior of tetraploid and diploid strains was very carefully studied under field conditions in an isolated plot, in order to exclude the possibility of contamination from plants other than those used in the experiment. In this crossing experiment special attention also was given to the selection of strains with combinations of genetic characters that would facilitate not only the determination of the amount of natural crossing but also the positive identification of the various possible types of progeny. Within the plot $4n$ and $2n$ plants were grown in alternate rows to insure abundant opportunity for natural cross-pollination. To verify the results obtained from the open-pollinated ears, controlled pollinations were made with separate and mixed samples of pollen from the two stocks.

The $4n$ stock selected for the crossing studies was a green plant type, lacking anthocyanin color ($A b pl r'$) and having nonliguleless leaves (Lg), nonyellow endosperm (y), and colorless aleurone ($A Cr$)⁵.

⁵ EMERSON, R. A. A FIFTH PAIR OF FACTORS, Aa , FOR ALEURONE COLOR IN MAIZE, AND ITS RELATION TO THE Cc AND Rr PAIRS. N. Y. (Cornell) Agr. Expt. Sta. Mem. 16, pp. 231-289, illus. 1918.

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The $2n$ stock was also a green plant type, but it had a genetic constitution ($a B pl R^+$) differing from that of the $4n$ stock, so that the F_1 hybrid would have sun-red plant color and colored anthers, phenotypically $A B pl R^+$ and readily distinguishable from both parent types. The

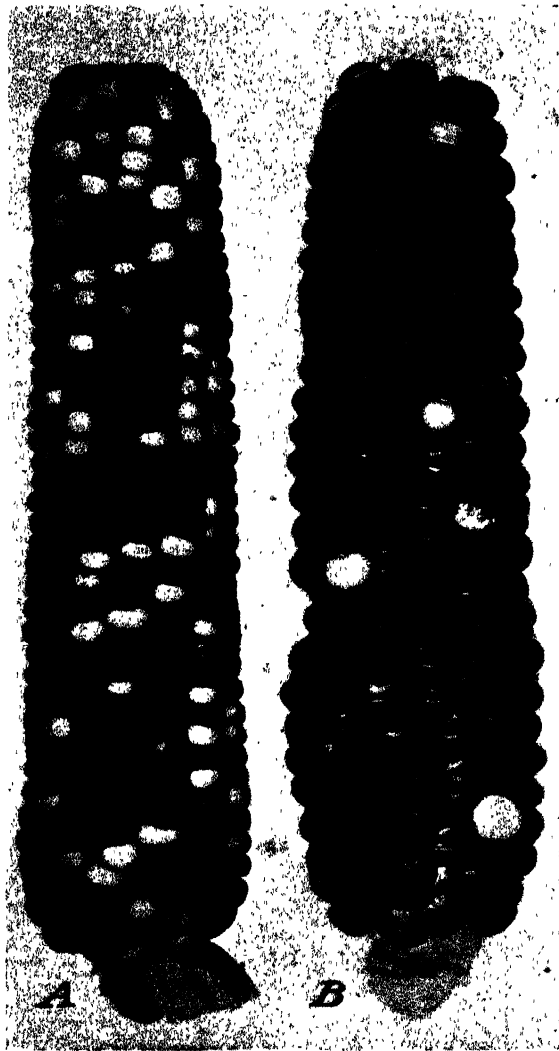


FIGURE 6.—Selfed ears of diploid (A) and tetraploid (B) maize plants heterozygous for the factor pair Rr illustrating segregations that approximate the expected 3 : 1 and 35 : 1 ratios.

$2n$ stock, moreover, was liguleless (lg), with yellow endosperm (Y) and colorless aleurone of a constitution ($a C R$) that would give colored aleurone and yellow endosperm in hybrid combinations with the $4n$ stock.

With these combinations of genes the selfed seed on both the $2n$ and $4n$ plants would be colorless and the hybrid seed colored. Assuming normal fertilization, the colorless seed would produce only green plants. Irregularities in the fertilization process, resulting in maternal embryos developed parthenogenetically or from heterofertilization,⁶ would be detectable as green plants of the parental types appearing among the hybrid sun-red plants from the colored seeds.

Representative ears of the parent stocks and their hybrids are illustrated in figure 7. Few fully developed kernels were produced on the cross-pollinated ears, the majority of the grains being only partially developed. This partial development, however, involved the endosperm as well as the pericarp, so that the defective hybrid grains could be distinguished from the nonhybrid grains on the basis of aleurone color. The results obtained from intercrossing the $2n$ and $4n$ stocks are summarized in table 1. The hybrid seeds both from the direct and the reciprocal cross were roughly classified as well filled and aborted. Many of the well-filled grains, i. e., grains with fully developed endosperms, were germless or had defective germs and failed to produce viable seedlings. In the cross $2n \times 4n$ only about 10 percent of the well-filled grains and very few of the aborted grains germinated. In the reciprocal $4n \times 2n$ cross nearly all of the well-filled grains and a large number of the aborted grains produced viable seedlings. All of the seedling progeny of these crosses were triploid with the exception of four tetraploid seedlings from one $2n$ ear. Presumably this ear had a $4n$ sector which produced $2n$ eggs, these in combination with normal $2n$ male gametes from the $4n$ pollen parent giving the exceptional tetraploids.

TABLE 1.—Intercrosses between diploid ($a C R^+ B-lg$) and tetraploid ($A C' r^+ b-lg$) strains of maize grown in an isolated plot and hand-pollinated

Type of cross	Ears pollinated	Hybrid (colored) seeds		Seedling progeny (sun-red, nonliguleless)	
		Well filled	Aborted	$3n$	$4n$
	Number	Number	Number	Number	Number
$2n \times 4n$	253	427	20,180	58	4
$4n \times 2n$	96	90	10,170	1,438

¹ Included in this number are 2 exceptional plants which were nonliguleless but lacked plant color.

All but 2 of the 500 F_1 seedlings from the $2n$ and $4n$ intercrosses were phenotypically sun-red and nonliguleless, indicating that they were true hybrids of constitution $Aa Bb-Lglg R^+ r^+$. Chromosome counts made from 150 of these sun-red nonliguleless seedlings, selected at random from the entire group of 498, showed that all were triploid. The 2 exceptional seedlings were nonliguleless and lacked plant color in the seedling stage. At maturity the glumes and anthers also were green, indicating the phenotypic constitution $A b Lg r^+$. Both of these plants were 30-chromosome triploids, as determined by root-tip counts, and their triploid nature was verified by pollen and stomata examinations at maturity. The absence of

⁶ SPRAGUE, G. F. THE NATURE AND EXTENT OF HETERO-FERTILIZATION IN MAIZE. Genetics 17: 358-368, illus. 1932.

the characters normally associated with the nonlinked dominant genes *B* and *R'* in these two plants is not understood. However, cogent cytogenetic data may be obtained from their progeny. No

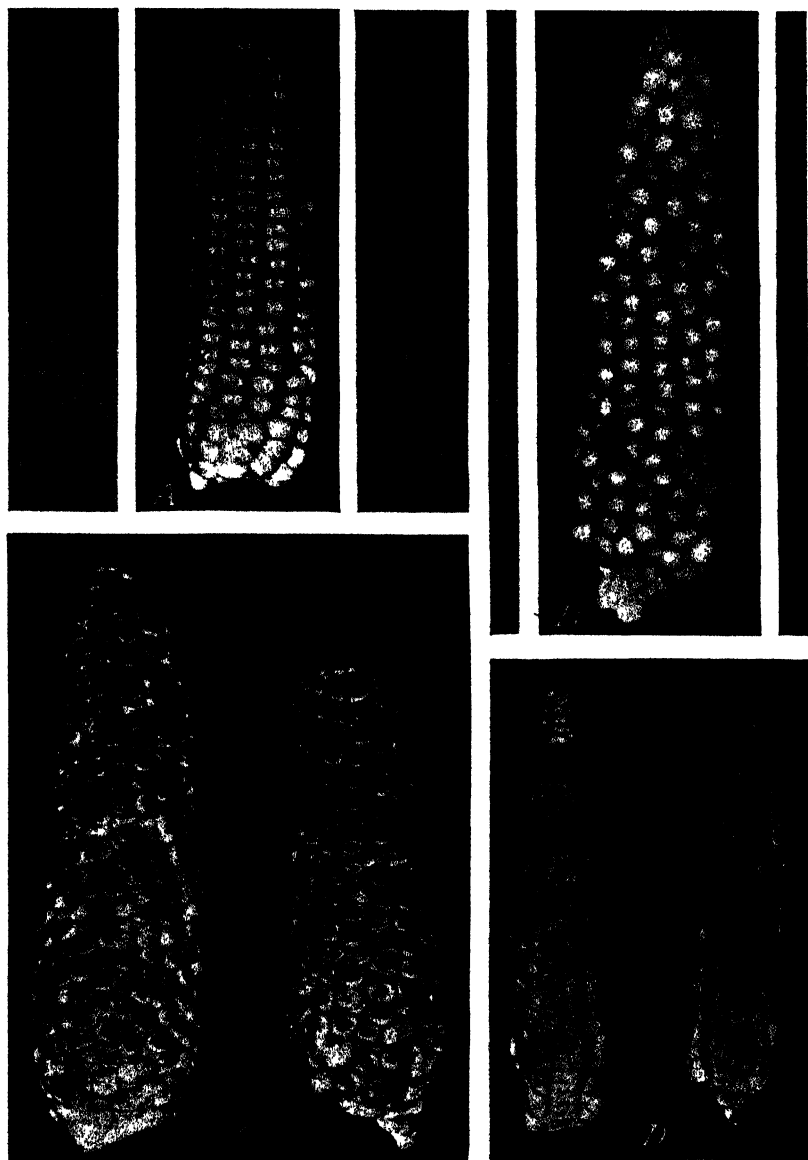


FIGURE 7.—Ears of diploid *a C R' B-Ig* (A) and tetraploid *A C r' b-Lg* (B) stocks and the direct $2n \times 4n$ (D) and reciprocal $4n \times 2n$ (C) crosses between them.

other cytogenetically detectable exceptional types, such as maternal monoploids or diploids, appeared in the $2n$ and $4n$ crosses.

The crossing behavior of the $2n$ and $4n$ stocks grown together in a mixed isolated planting and open-pollinated was compared with con-

trolled pollination. Approximately equal amounts of pollen from the $2n$ and $4n$ stocks were thoroughly mixed and applied to both the $2n$ and $4n$ parent plants in the isolated plot. The results are summarized in table 2. Representative ears showing the set of seed obtained from these mixed pollinations are illustrated in figure 8. In this, as in the former experiment, the selfed seeds were colorless and the crossed seeds were colored.

TABLE 2.—Intercrosses between diploid ($a C R^+ B-lg$) and tetraploid ($A C r^+ b-Lg$) strains of maize grown together in an isolated plot, comparing open pollinations and hand pollinations with mixtures of $2n$ and $4n$ pollen

Type of cross	Ears pollinated	Crossed seed (colored)		Selfed seed (non- colored)
		Well filled	Aborted	
	Number	Number	Number	Number
$2n \times (2n+4n)$	77	5	103	9,938
$4n \times (2n+4n)$	26	60	3,324	467
$2n$ (open pollinated)	105	7	23	18,200
$4n$ (open pollinated)	81	79	8,311	8,148

In the cross $2n \times (2n+4n)$ the pollen of the diploid functioned almost to the exclusion of the $4n$ and produced uniformly well-filled ears of colorless selfed seed with only an occasional colored hybrid grain (fig. 8, A). The cross $4n \times (2n+4n)$ produced chiefly hybrid grains most of which were aborted; relatively few (5 to 10 percent) selfed seed were formed when the cross was made with the tetraploid as pistillate parent (fig. 8, B). Similar results were obtained with the open-pollinated ears. Most of the $4n$ ears showed considerable evidence of hybridization, the ratio of selfed to hybrid seed probably being dependent in large measure upon the relative amounts of $2n$ and $4n$ pollen available when the silks were exposed (fig. 8, C). However, the diploid ears under similar conditions showed little or no evidence of hybridization (fig. 8, D). The mixed open and closed pollinations recorded in table 2 involved 289 ears, which produced 48,665 kernels. From the hybrid colored seed there were obtained 214 seedlings, of which 16 were from diploid ears and the remainder from the tetraploid ears. All of these seedlings were triploid hybrids. The frequency with which hybrid seedling progeny resulted from the mixed pollinations was less than 1 percent.

These results indicate that $2n$ and $4n$ strains of maize are highly cross-sterile. Furthermore the triploid progeny of these crosses are themselves highly sterile (fig. 9), and yield mostly defective plants with unbalanced chromosome numbers. Thus tetraploid maize can be maintained under natural field conditions without hybridizing effectively with diploid stocks, a fact of major practical importance if it is found that tetraploid lines can be utilized advantageously in the production of hybrid seed corn.

The high degree of incompatibility exhibited by the maize tetraploids in crosses with normal diploid stocks obviously is to be attributed to quantitative rather than qualitative differences—i. e., to chromosome number differences—since the derived $4n$ stocks obtained in the experiments had a genetic constitution either very similar to or identical with that of the parental diploid stocks. Triploid

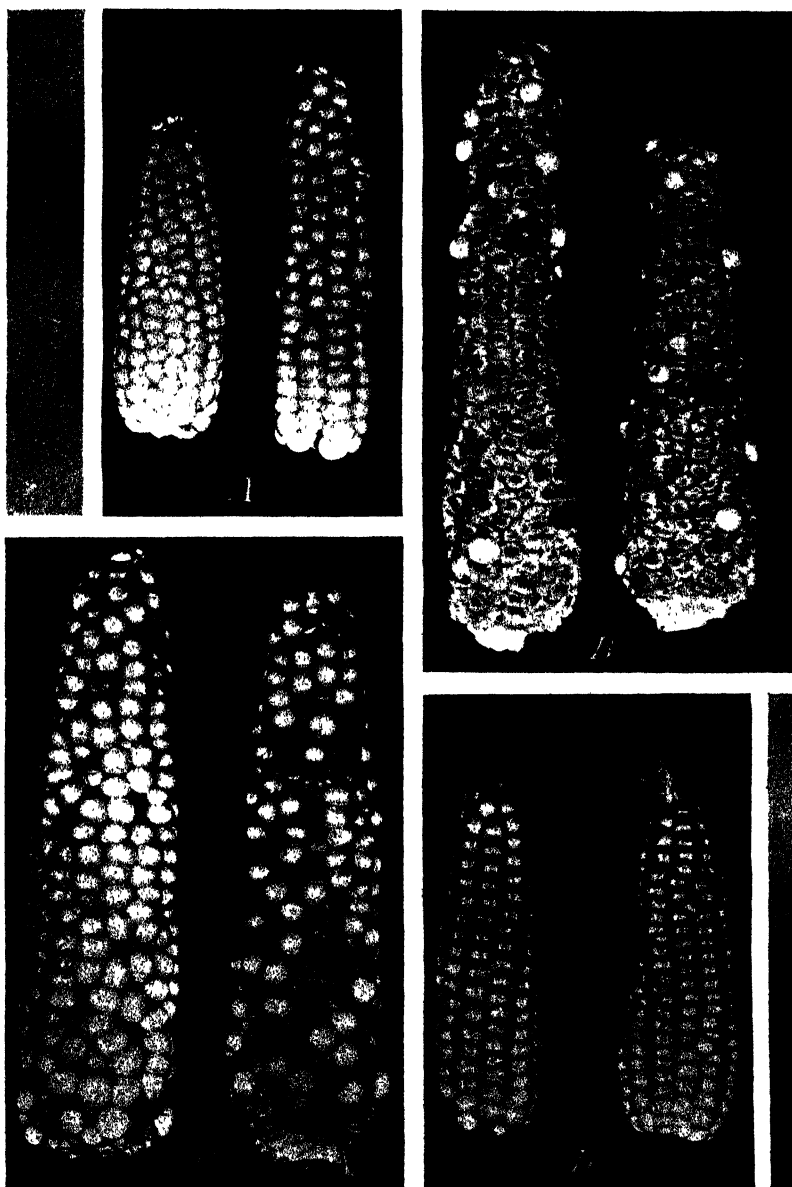


FIGURE 8.--Ears illustrating the set of seed obtained from intercrossing diploid aCR (A) and tetraploid $A Cr$ (B) stocks with mixtures of pollen from the two parents; also open-pollinated tetraploid (C) and diploid (D) ears of these same stocks grown together in an isolated plot.

hybrids were obtained with a low frequency in both direct $2n \times 4n$ and reciprocal $4n \times 2n$ crosses, the latter yielding a significantly higher frequency. Ineffectual pollen-tube growth is excluded as a cause of this incompatibility, since fertilization took place and kernel development uniformly was initiated, as evidenced by the presence of aborted

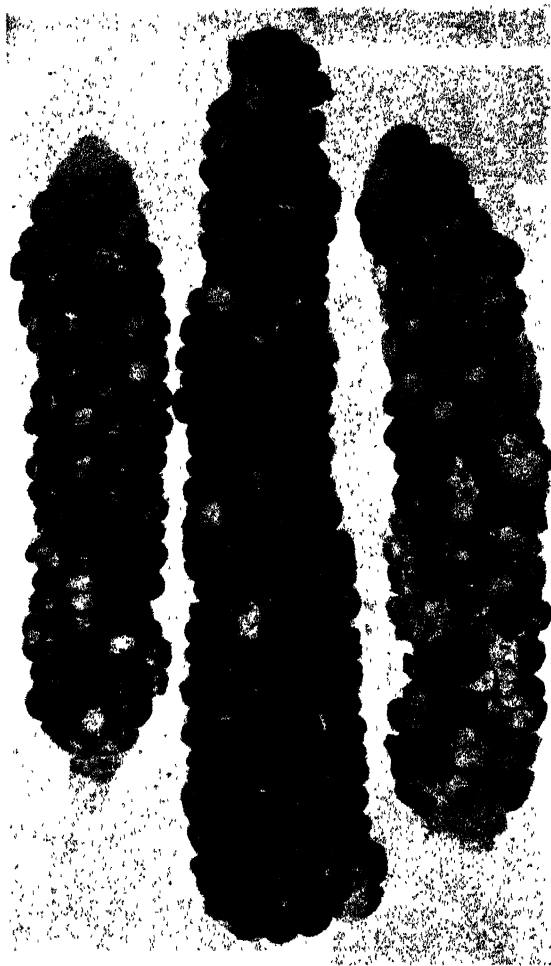


FIGURE 9 —Typical ears of self-pollinated triploid maize produced by intercrossing diploids and tetraploids.

hybrid grains. In fact, the results of the mixed and open $4n \times (2n + 4n)$ pollinations demonstrated that the pollen of the diploid actually functioned on the $4n$ plants almost to the complete exclusion of the pollen of the tetraploid.

None of the current hypotheses advanced to account for the arrested seed development, or so-called "hybrid incompatibility" between autotetraploids and the parental diploids, when considered individually, offers a wholly satisfactory explanation of the situation in maize. It is possible that changed chromosome relationships between the embryo and endosperm, or between the embryo, endosperm, and

seed-parent tissue; or different strengths of activating stimuli in the male gametes, correlated with chromosome number and influencing the rate of growth of the embryo and endosperm, may account for part but certainly not for all of the observed facts pertaining to the direct and reciprocal crosses and to the occurrence of spontaneous triploids.

Noteworthy is the fact that the nonoccurrence of spontaneous tetraploids may be better understood in view of the observed inability of $2n$ pollen grains produced by tetraploids to compete successfully with the n pollen normally produced by diploids.

CHROMOSOME BEHAVIOR

The 40 chromosomes of tetraploid maize were arranged at the metaphase of the first meiotic mitosis usually in quadrivalent and bivalent groups exclusively (fig. 10, A). Trivalents and univalents

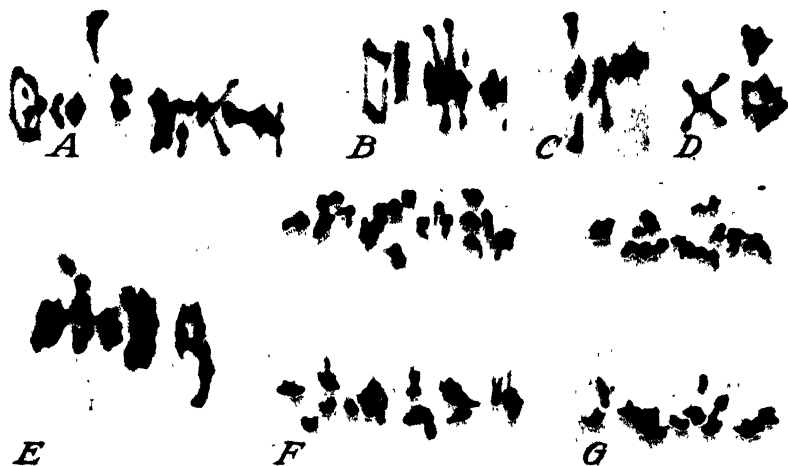


FIGURE 10. — Photomicrographs of the chromosomes of tetraploid maize illustrating certain phases of the first meiotic or reduction division. A, Metaphase I with quadrivalent and bivalent associations; B-D, early anaphases with adjacent (B and D) and alternate (C) members of certain quadrivalents passing to the same pole; E, precocious disjunction of one quadrivalent with adjacent members still attached; F, typical anaphase I with regular assortment of daughter chromosomes, G, anaphase I with a single lagging univalent chromosome.

were noted very rarely, the frequency being 11 out of a total of 402 metaphases observed. Most frequently the number of quadrivalents varied from 7 to 9, and the number of bivalents varied correspondingly from 3 to 1. Characteristic types of quadrivalent associations are illustrated in figure 10, A, D, the arrangements being of such a nature that either adjacent (fig. 10, B) or alternate (fig. 10, C) chromosomes pass to the same pole in the anaphase. In the former case the adjacent chromosomes passing to the same pole frequently were not separated from one another until they were some distance from the equatorial plate region. The anaphase figures usually were regular, with 20 chromosomes passing to one pole and 20 to the other (fig. 10, B). In 14 of 21 anaphases counted, each group contained 20 chromosomes; in 6 cases the disjunction was 19 to 21 and in one case it was 18 to 22. A search was made for lagging univalent chromosomes in the mid and late anaphase and early telophase stages;

13 cases of a single lagging univalent (fig. 10, *G*) and 2 cases of 2 lagging univalents were noted among 348 such figures. In a survey of the first divisions in the microspore, 16 spores were found with 20 chromosomes, 5 with 19, 2 with 18, 4 with 21, and 1 with 22 chromosomes.

The observations on chromosome distribution at meiosis and the microspore counts suggested that the tetraploids probably would not breed true for chromosome number. The following counts were made from seedling root tips of plants selected at random from among the selfed progeny of individuals known to have 40 chromosomes:

Chromosome number:	Number of individuals
37-----	1
38-----	3
39-----	6
40-----	27
41-----	12
42-----	5

Half of the 54 plants counted had 40 chromosomes like the parent; the others had numbers other than 40, ranging from 37 to 42. From an examination of these plants at maturity there appeared to be no definite positive correlation between off-type chromosome number and deficient vigor. These data were obtained from first- and second-generation tetraploids. Whether the tendency to breed true for chromosome number tends to become more or less variable in subsequent generations is not known.

SUMMARY AND CONCLUSIONS

Tetraploid maize (*Zea mays* L.) closely resembles normal diploid maize in height and habit of growth. But the individual organs of tetraploid plants are larger than those of the parental diploids; broader leaves, sturdier stalks, larger tassels, and ears and kernels of increased size characterize the tetraploids.

An increase in cell size with no apparent diminution in chromosome size accompanies chromosome doubling in maize, with the result that the pollen, epidermal cells, and stomata, as well as the cells in other parts of $4n$ plants, are proportionately larger than those of the diploid plants from which they were derived.

The tetraploid strains of maize thus far produced are not as fertile as the original diploids, the ears ordinarily being not entirely well filled. Taking the set of seed as a measure of fertility, the amount of reduced fertility varies from 5 to 20 percent in different $4n$ stocks.

Diploid maize and tetraploid maize exhibit marked incompatibility and are highly cross-sterile in both direct and reciprocal crosses. When the diploid was used as the seed parent the amount of fertility as measured by the proportion of viable to nonviable seeds was less than 0.5 percent. In the reciprocal cross the amount was somewhat higher, ranging from 3 to 5 percent. The resulting progeny with rare exceptions were triploid hybrids.

The pollen of tetraploid plants is unable to compete favorably with that of $2n$ plants, either on its own silks or on the silks of diploids, as indicated by the results of open and controlled pollinations in an isolated mixed planting of $2n$ and $4n$ stocks.

Tetraploid maize can be maintained under natural field conditions together with ordinary diploid maize without an appreciable amount of hybridization.

The high degree of incompatibility exhibited by tetraploid maize in crosses with diploid maize is to be attributed to quantitative rather than to qualitative chromosomal differences, since the induced $4n$ stocks had the same or very similar kinds of genes as the parental diploids, with which they subsequently proved to be highly cross-sterile.

Tetraploid stocks breed true for tetraploidy, no reversions to diploidy having been observed during several generations of breeding.

Variations in chromosome number involving one or a few chromosomes more or less than the typical number 40 occur in the progeny of tetraploids. This variation in number apparently has little or no effect on the appearance of the plants. There was no definite positive correlation between atypical chromosome number and deficient vigor or unusual habit of growth.

In meiosis the association of the chromosomes was almost exclusively in quadrivalents and bivalents, with usually 7 to 9 quadrivalents and 3 to 1 bivalents; trivalents were rarely seen. Irregularities in meiotic chromosome distribution were noted which would account for the observed inconstancy of number in the progeny of tetraploids.

COMPARATIVE RESISTANCE OF FIVE BREEDS OF CHICKENS TO THE NEMATODE ASCARIDIA LINEATA (SCHNEIDER)¹

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INTRODUCTION

Experimental evidence of the resistance of chickens to the nematode *Ascaridia lineata* (Schneider) was obtained in 1921 by Ackert and Herrick (9)² while studying the effects of this intestinal parasite on chickens. This finding led to studies of the factors influencing this resistance. Among the significant factors were found to be the presence of vitamins A and B, certain dietary supplements, loss of blood, previous infestations, and age. The significance of vitamin A was reported by Ackert, McIlvaine, and Crawford (10); that of vitamin B, by Ackert and Nolf (12); that of certain dietary supplements, by Ackert and Beach (4); and that of loss of blood, by Ackert (1) and by Porter and Ackert (25). Graham, Ackert, and Jones (18) found that resistance was influenced by previous infestations of *A. lineata*, and Herrick (20) and Ackert, Porter, and Beach (13) that age was a factor. All these studies were made on purebred Single-Comb White Leghorns. As 2-month-old chicks of this breed were found by Ackert (2) to be very susceptible to *A. lineata*, it seemed desirable to ascertain whether other breeds of chickens were more resistant to this nematode than White Leghorns, or less.

Accordingly, experiments were begun in 1932 to compare the resistance of Single-Comb White Leghorns to *Ascaridia lineata* with that of Single-Comb Rhode Island Reds, Barred Plymouth Rocks, White Plymouth Rocks, Buff Orpingtons, and White Minorcas.

MATERIALS AND METHODS

The chickens used in these experiments were secured as day-old chicks from the same commercial hatchery and raised in confinement under conditions found by Herrick, Ackert, and Danheim (21) to be suitable for normal growth and development. In these experiments the following ration was used: Yellow corn meal, 40 g; ground oats, 17 g; alfalfa leaf meal, 4 g; meat meal, 10.4 g; powdered milk, 6.4 g; cracked wheat, 15 g; and cod-liver oil, 1.69 g. In order that the vitamin A potency of the cod-liver oil might be retained, a barrel of feed sufficient for 300 chickens was mixed each week.

The nematode eggs for parasitizing were secured from adult live worms of approximately the same length. The egg cultures were prepared in the following manner: The end of a worm was excised, and the internal organs were pressed into a sterile Petri dish; the uteri were isolated and transferred to another sterile dish. At various

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² Reference is made by number (italic) to Literature Cited, p. —.

points the uteri were punctured for the liberation of eggs with characteristic light centers, shown by Ackert (3) to be fertile. The portions of uteri containing fertile eggs were transferred to a final sterile Petri dish, and the eggs pressed out and covered with sterile distilled water. Four or five drops of 2-percent formalin were added to prevent bacterial or fungous growth. The cultures were incubated approximately 3 weeks at 27° to 30° C.

When the chicks were 9 days old, they were banded, divided into groups, and each chick weighed. Weekly weights were taken thereafter until the termination of the experiment. Group A was parasitized at 12 days of age, group B at 19 days, group C at 30 days, group D at 33 days, and group E at 44 days. In parasitizing, each chicken of a group was given 50 ± 5 embryonated eggs of *Ascaridia lineata* as Ackert, Graham, Nolf, and Porter (7) found this number suitable for comparative infestations of the worms. The eggs were counted with the aid of a compound microscope and a mechanical stage. The drop of water containing the correct number of eggs was wiped off the slide with a small piece of filter paper, which was placed in the chick's esophagus with forceps.

After 3 weeks of parasitism the chickens were killed, the intestines removed, and the contents flushed out quickly by the hydraulic method of Ackert and Nolf (11). The nematodes were preserved in 10-percent formalin. In measuring, the shadow of each worm magnified six times, was thrown on the ground glass of a photographic bellows and traced on onionskin paper; from these tracings direct measurements were made with a calibrated, milled wheel.

The criteria adopted in making statistical comparisons of resistance among the different breeds of chickens to *Ascaridia lineata* were the number of worms and their length from each group of chickens under comparison.

EXPERIMENTAL DATA

GROWTH OF THE CHICKENS

In the various experiments 1,351 chickens were used. The average weights taken at 9 days of age showed the White Leghorns and White Minorcas, both Mediterranean breeds, to be the heaviest, slightly over 62 g each. The heavier breeds and varieties averaged distinctly less: The Rhode Island Reds, 53.6 g; the Barred Plymouth Rocks, 54.6 g; the White Plymouth Rocks, 57.1 g; and the Buff Orpingtons, 57.7 g (fig. 1). All the chickens were kept under the same conditions and were given the same ration, which was kept constantly before them. At the close of the first week of the experiment, the White Plymouth Rocks averaged the heaviest and the Barred Plymouth Rocks the lightest, the White Minorcas taking a lower rank and the Rhode Island Reds and Buff Orpingtons making more rapid gains. In 2 weeks, when the chicks were 30 days old, the White Plymouth Rocks were the heaviest, the White Leghorns next, the Buff Orpingtons and Rhode Island Reds following them, with the White Minorcas and Barred Plymouth Rocks making the least rapid gains. During the next 2 weeks the White Leghorns' rate of gain decreased and that of each of the heavier breeds or varieties, the White Plymouth Rocks, Buff Orpingtons, Rhode Island Reds, and Barred Plymouth Rocks, made better gains. The White Minorcas continued to make the slowest increase in weight (fig. 1).

At 51 days of age a slight break may be noticed in the growth rates of some of the breeds or varieties of chickens.

This result is in accord with that of Brody (14), who found two characteristic growth cycles in the post-embryonic life of domestic chickens, one of which climaxed at about 8 weeks of age.

After about a week, when the chickens were 58 days of age, an increased growth rate again appeared in all 5 breeds or varieties (fig. 1). At that time the White Leghorns and White Minorcas were definitely separated in their growth rates from the 3 other breeds or varieties, and 1 week later the White Leghorns had the slowest growth and the White Minorcas next. Of the 4 heavier breeds or varieties, the White Plymouth Rocks continued to lead and the Buff Orpingtons to trail in growth.

At the close of the experiment (end of the seventh week), when the chickens were 63 days of age, the White Plymouth Rocks were slightly the heaviest and the Rhode Island Reds next. Third and fourth in weight, respectively, were the Barred Plymouth Rocks and Buff Orpingtons. The White Minorcas, a breed shown by Jull (22) to be somewhat heavier than the White Leghorns, were making rapid growth (fig. 1).

The weights of these chickens grown in confinement but with ample light and air are very similar to those of corresponding breeds raised by Warren (33) at the Kansas State College Poultry Farm. Warren made weekly records of the weights of White Leghorns, Rhode Island Reds, and Barred Plymouth Rocks for 10 weeks, beginning with day-old chicks. At 6 weeks of age the weights of the White Leghorns and Rhode Island Reds in his and in the present experiments were almost identical. His Barred Plymouth Rocks, however, were somewhat smaller, averaging 274.2 g as compared with 334.6 g in the present experiments. Similar weights were recorded by Kempster (23) and by Card and Kirkpatrick (15) for corresponding breeds and varieties of chickens at 6 weeks of age.

THE RESISTANCE OF WHITE LEGHORNS COMPARED WITH THAT OF THE OTHER BREEDS AND VARIETIES

GROUP A, 33 DAYS OF AGE

The experiments on resistance were conducted in five groups, A to E. In group A, the chickens, when 12 days of age, were parasitized with 50 ± 5 eggs of the nematode *Ascaridia lineata*. After the 3-week period of parasitism all chickens of the group were killed, the worms from each bird counted and measured, and the data for each breed or variety assembled.

In table 1 it is seen that from the White Leghorns there was an average of 8.28 worms per bird and from the Rhode Island Reds an average of 4.89 worms, a difference of 3.39 worms which was 3.03 times the probable error and was therefore considered to be significant. In comparing the length of the worms from these two groups it was found that those from the Leghorns averaged 22.08 mm and the ones from the Reds 17.66 mm, a significant difference of 4.42 mm. The results of this test indicate that the Rhode Island Reds were significantly more resistant to *Ascaridia lineata* than were the White Leghorns.

The data in table 1 for the 36 White Plymouth Rocks indicate that they were more resistant than the Leghorns to the growth of the nematodes.

The Buff Orpingtons in this group proved to be just as susceptible as were the White Leghorns to *Ascaridia lineata*, the differences in number and in length having been negligible.

No constant difference was found between the number of nematodes from the Barred Plymouth Rocks and that from the Leghorns, but on comparing the length of the worms it was found that the worms

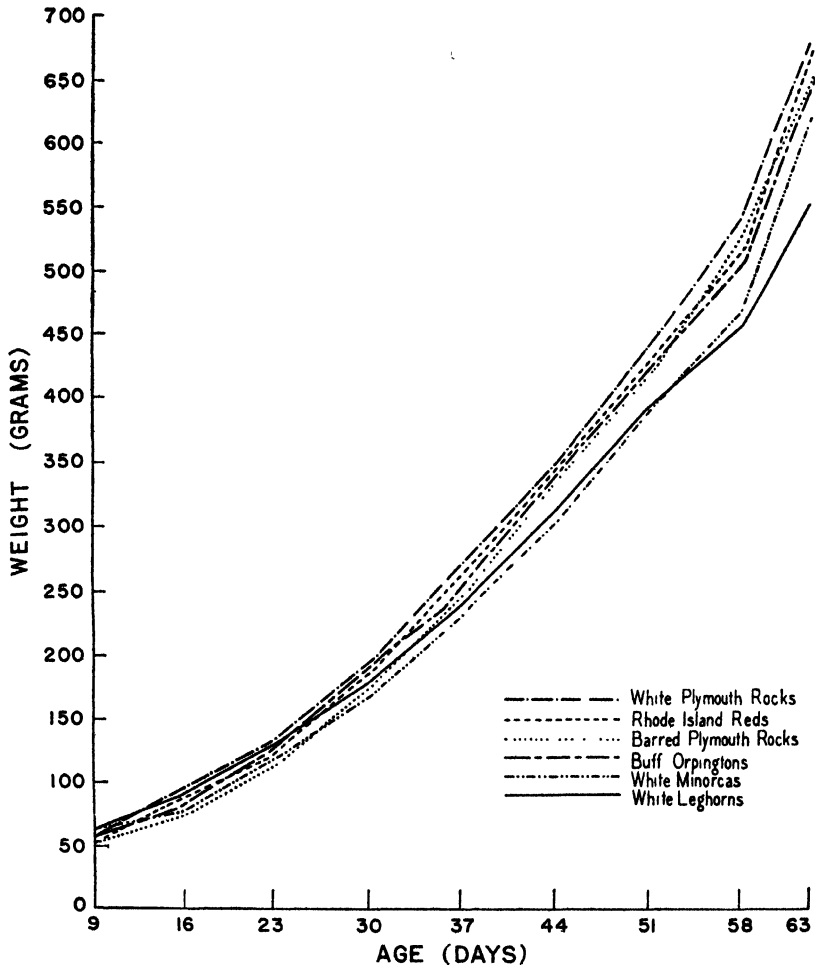


FIGURE 1.—Growth curves of 5 breeds and 2 varieties of young chickens from 9 to 63 days of age.

from the Leghorns averaged 2.80 mm longer, a significant difference (8.12 times the probable error). This comparison again showed that the Leghorns were less resistant to the growth of these nematodes.

The comparison of the worms from the White Minorcas with those from the Leghorns was very similar to the previous comparison; no constant difference occurred in the number of worms, but those from the White Leghorns were 1.68 mm longer. This was a significant difference (table 1).

TABLE 1.—*The resistance to nematodes of White Leghorns as compared with that of 3 other breeds and 2 varieties of a fourth breed of chickens, as determined by the number and length of Ascaridia lineata recovered from each breed at autopsy.*

GROUP A, PARASITIZED AT 12 DAYS OF AGE AND EXAMINED AT 33 DAYS

Breed	Hosts	Worms	Difference	Ratio, $\frac{D^1}{E}$	Mean length of worms	Difference	Ratio, $\frac{D}{E}$
	Num- ber	Mean number			Mm		
White Leghorns	46	8.28	3.39	3.03	22.08	4.42	10.83
Rhode Island Reds	35	4.89			17.66		
White Leghorns	46	8.28	1.20	.90	22.08	1.74	3.24
White Plymouth Rocks	36	7.08			20.34		
White Leghorns	46	8.28	.91	.60	22.08	.36	1.12
Buff Orpingtons	36	9.19			22.44		
White Leghorns	46	8.28	.94	.63	22.08	2.80	8.12
Barred Plymouth Rocks	36	9.22			19.28		
White Leghorns	46	8.28	.81	.72	22.08	1.68	4.22
White Minorcas	32	7.47			20.40		

GROUP B, PARASITIZED AT 21 DAYS OF AGE AND EXAMINED AT 40 DAYS

White Leghorns	61	7.34	3.73	4.79	18.19	3.73	8.71
Rhode Island Reds	51	3.61			14.46		
White Leghorns	61	7.34	4.90	7.34	18.19	4.82	5.46
White Plymouth Rocks	55	2.44			13.37		
White Leghorns	61	7.34	3.92	5.38	18.19	.81	1.56
Buff Orpingtons	50	3.42			17.38		
White Leghorns	61	7.34	3.63	5.07	18.19	1.41	2.97
Barred Plymouth Rocks	48	3.71			16.78		
White Leghorns	61	7.34	.66	.72	18.19	2.12	6.63
White Minorcas	40	8.00			20.31		

GROUP C, PARASITIZED AT 30 DAYS OF AGE AND EXAMINED AT 51 DAYS

White Leghorns	49	5.73	2.89	3.79	23.83	3.65	6.78
Rhode Island Reds	43	2.84			20.18		
White Leghorns	49	5.73	2.34	2.99	23.83	3.37	7.61
White Plymouth Rocks	61	3.39			20.46		
White Leghorns	49	5.73	.48	.49	23.83	.09	.21
Buff Orpingtons	53	5.25			23.92		
White Leghorns	49	5.73	3.02	4.14	23.83	4.19	6.22
Barred Plymouth Rocks	52	2.71			19.64		
White Leghorns	49	5.73	3.22	4.53	23.83	4.80	9.72
White Minorcas	49	2.51			19.03		

GROUP D, PARASITIZED AT 33 DAYS OF AGE AND EXAMINED AT 54 DAYS

White Leghorns	51	1.22	0.36	1.18	20.00	3.41	4.61
Rhode Island Reds	59	1.58			16.59		
White Leghorns	51	1.22	.07	1.80	20.00	3.07	3.71
White Plymouth Rocks	61	1.15			16.93		
White Leghorns	51	1.22	.49	1.62	20.00	2.06	2.52
Buff Orpingtons	52	1.71			17.94		
White Leghorns	51	1.22	.05	.02	20.00	2.08	2.57
Barred Plymouth Rocks	51	1.27			17.92		
White Leghorns	51	1.22	.13	.40	20.00	3.74	4.37
White Minorcas	26	1.35			23.74		

GROUP E, PARASITIZED AT 44 DAYS OF AGE AND EXAMINED AT 65 DAYS

White Leghorns	46	2.00	0.64	1.73	21.45	3.87	4.03
Rhode Island Reds	36	1.86			17.58		
White Leghorns	46	2.00	.30	.83	21.45	1.53	2.45
White Plymouth Rocks	37	1.70			19.92		
White Leghorns	46	2.00	1.00	2.99	21.45	5.57	5.57
Buff Orpingtons	21	1.00			15.88		
White Leghorns	46	2.00	1.31	3.99	21.45	4.03	3.91
Barred Plymouth Rocks	35	.69			17.42		
White Leghorns	46	2.00	.81	2.33	21.45	3.74	5.62
White Minorcas	48	1.19			17.71		

¹ The ratio as here used is the quotient of the difference divided by its probable error.

In this experiment the Leghorns were significantly less resistant to the growth of the worms in 4 of the 5 comparisons.

GROUP B, 40 DAYS OF AGE

In the group B series of experiments the chickens were parasitized at 21 days of age and examined when 40 days old.

In both number and length of worms the White Leghorns were constantly less resistant to the parasites than the Rhode Island Reds (table 1).

On comparing *Ascaridia lineata* from the White Rocks with those from the Leghorns an average of 4.90 more was found in the Leghorns, a difference 7.34 times the probable error. The worms from the White Leghorns were also significantly longer than those from the White Rocks, the difference being 5.46 times the probable error. Here again were found constant differences in the number and in the length of worms, which indicated that the White Leghorns were less resistant to *A. lineata* than were the White Plymouth Rocks.

From the Buff Orpingtons there was recovered an average of 3.92 worms less than from the White Leghorns, a significant difference. There was but slight difference between the two breeds in length of worms. This result is similar to that obtained in the comparison of Buff Orpingtons and White Leghorns in group A.

In comparing the Barred Plymouth Rocks with the White Leghorns the latter were found to be constantly less resistant to these intestinal worms (table 1).

The average number of worms in the White Minorcas was approximately the same as that in the White Leghorns, while the average length was greater in the Minorcas than in the Leghorns.

Many of the evidences from this series of experiments point to the White Leghorns as being less resistant to *A. lineata* than were the other breeds and varieties of chickens.

GROUP C, 51 DAYS OLD

The chickens of the third group were parasitized at the age of 30 days. They were examined when 51 days old.

The White Leghorns again averaged more and longer worms than the Rhode Island Reds. This, together with the results of the previous tests, points to the White Leghorns as being less resistant to these nematodes.

The White Plymouth Rocks showed an average of 2.34 worms less than those in the White Leghorns, a difference which is probably significant (2.99 times the probable error). The White Plymouth Rocks were more resistant also to the growth of *Ascaridia lineata*, the worms from them averaging 3.37 mm less in length than those from the White Leghorns, a significant difference.

As in the two previous groups, no constant differences either in the number or in the length of *A. lineata* were noted between the Buff Orpingtons and the White Leghorns.

Both as to number and length of *Ascaridia lineata*, the White Leghorns were less resistant than were the Barred Plymouth Rocks.

From the White Minorcas 3.22 fewer worms were isolated than from White Leghorns, a difference which was significant; the worms were also shorter by 4.80 mm on an average.

Results in this group gave further evidence that the White Leghorn chickens were less resistant to *A. lineata* than were the other breeds and varieties of chickens.

GROUP D, 54 DAYS OF AGE

The chickens in group D were parasitized at 33 days of age and examined when 54 days old.

Approximately the same average number of *Ascaridia lineata* were isolated from the Rhode Island Reds as from the White Leghorns. However, the worms from the Leghorns averaged 3.41 mm longer, a difference 4.61 times the probable error.

Only slight differences in the average number of worms were found between the White Plymouth Rocks and the White Leghorns, but the worms from the Leghorns averaged 3.07 mm longer than those from the White Plymouth Rocks, a significant difference. In this comparison, as in that between the length of worms from the Rhode Island Reds and from the White Leghorns, the evidence indicates that the White Leghorns were less resistant than either the Reds or the White Rocks to the growth of *Ascaridia lineata* in their digestive tracts.

In the fourth consecutive experiment no constant differences were found between the number and the length of the worms from the Buff Orpingtons and those from the White Leghorns. The *A. lineata* averaged somewhat longer in the Leghorns in this group, but the difference was not significant.

Similar results were obtained in the comparison of the number and the length of the *Ascaridia lineata* from the Barred Plymouth Rocks and those from the White Leghorns. With a larger number of hosts it is probable that the average difference in the length of *A. lineata* in these two breeds would have been significant, as the worms averaged 2.08 mm longer in the White Leghorns, a difference 2.57 times the probable error. Essentially the same average number of *A. lineata* was found in the White Minorcas as in the White Leghorns. The Minorcas were less resistant, as the worms averaged 3.74 mm longer than those from the Leghorns. While there were no essential differences in number of worms isolated from chickens in this group; those from the Leghorns had the greater average length in 4 of the 5 comparisons.

GROUP E, 65 DAYS OF AGE

The fifth experiment was performed on chickens parasitized at 44 days of age and examined when 65 days old.

On counting the worms it was found that the difference between the number of worms isolated from the White Leghorns and from the Rhode Island Reds was negligible, but those from the White Leghorns were 3.87 mm longer on an average than those from the Rhode Island Reds, a significant difference. In this test the White Leghorns had more worms and longer ones, indicating that the Leghorns were less resistant to *Ascaridia lineata* than were the Reds.

Only a slight difference was found between the average number of worms from the White Plymouth Rocks and that from the White Leghorns. The length of the worms was somewhat greater in the Leghorns, and with a larger number of hosts the difference would probably have proved significant.

Slightly fewer nematodes were found in the Buff Orpingtons than in the Leghorns. These heavy Buff Orpingtons proved to be much more resistant than the Leghorns to the growth of the *Ascaridia lineata* as the worms from the Buff Orpingtons averaged 5.57 mm shorter, a significant difference.

The *A. lineata* from the Barred Plymouth Rocks reached the low average of 0.69 worm, an average of 1.31 worms less than that from the Leghorns. This proved to be a significant difference. The nematodes from the Leghorns also averaged 4.03 mm longer than those from the Barred Plymouth Rocks, a significant difference. Here again the indication is that the White Leghorns were less resistant than the heavy Barred Rocks.

The number of *Ascaridia lineata* from the White Minorcas was not significantly different from that from the Leghorns, although the Leghorns had the larger average number of worms. But the worms averaged 3.74 mm longer in the White Leghorns. This difference being significant gives evidence in 3 of the 5 comparisons of length of worms that the White Leghorns were less resistant to the growth of these intestinal worms than were the White Minorcas.

When the average number and length of the *Ascaridia lineata* from the White Leghorns are compared with the number and length of those from the other breeds and varieties of chickens in the five groups of experiments it is found that in 39 of the 50 comparisons the White Leghorns had more or longer worms and that the differences were significant in 25³ of the 39 times. Thus, the evidence from these five groups of experiments points unmistakably to the White Leghorns as being less resistant to these intestinal worms both in respect to number and size, than were any of the other breeds or varieties.

Why these fowls should be less resistant is not certain. They mature somewhat earlier than do the other breeds. Likewise, the plumage develops more rapidly in the Leghorns. These characteristics should tend to increase the resistance of the White Leghorns. The only marked difference observable between the White Leghorns and the other breeds was their greater activity in the pens. It is possible that in this greater utilization of nervous energy the White Leghorns correspondingly reduce their resistance to these intestinal parasites.

The fact that in two comparisons the White Minorcas were significantly more susceptible than the Leghorns indicates a possibility of different strains of White Minorcas. The worms from the White Leghorns in group C were somewhat longer than usual, and those from the White Minorcas in group E somewhat shorter, although, of course, the chickens were older and this difference may have been due to age resistance. The worms from the White Minorcas in group D averaged 23.74 mm, which was the largest average worm length from any group of the Minorcas. The differences may have been due to different strains among the *Ascaridia lineata*, but these cultures were made from worms of the same length and frequently consisted of the eggs from one worm. As the cultures were well mixed, one would expect to find longer average worms also in some of the other breeds in group D, but in all cases the average worm length was less in the other breeds or varieties in group D than in the White Minorcas or in the corresponding breeds in group C, which were younger (table 1).

³ This does not include three comparisons with ratios of 2.97 or 2.99 that were probably significant.

The evidence therefore is indicative of the occurrence of two strains of White Minorcas, one more susceptible than the other.⁴

COMPARATIVE RESISTANCE OF THE OTHER BREEDS AND VARIETIES TO
ASCARIDIA LINEATA

Consideration will next be given to the comparative resistance of the 3 breeds and 2 varieties other than White Leghorns as determined by the average number and length of *Ascaridia lineata* obtained from each. The data from the five experiments are given in table 2. As both the number and the length of the nematodes are considered in each group, there are 10 possible comparisons per group for each breed or variety; and in the five groups a total of 50 comparisons.

TABLE 2.—The comparative resistance to nematodes of 3 breeds and 2 varieties of a fourth breed of chickens as determined by the number and length of the *Ascaridia lineata* recovered from each breed at autopsy

GROUP A, EXAMINED AT 33 DAYS							
Breed	Hosts	Worms	Difference	Ratio, D ¹ E	Mean length of worms	Difference	Ratio
	Number	Mean number			Mm		
Rhode Island Reds	35	4.89	2.19	2.05	17.66	2.68	4.43
White Plymouth Rocks	36	7.08			20.34		
Rhode Island Reds	35	4.89	4.30	3.28	17.66	4.78	11.25
Buff Orpingtons	36	9.19			22.44		
Rhode Island Reds	35	4.89	4.33	3.41	17.66	1.62	3.66
Barred Plymouth Rocks	36	9.22			19.28		
Rhode Island Reds	35	4.89	2.58	3.17	17.66	2.74	5.65
White Minorcas	32	7.47			20.40		
White Plymouth Rocks	36	7.08	2.11	1.42	20.34	2.10	3.81
Buff Orpingtons	36	9.19			22.44		
White Plymouth Rocks	36	7.08	2.14	1.48	20.34	1.06	1.88
Barred Plymouth Rocks	36	9.22			19.28		
White Plymouth Rocks	36	7.08	.39	.36	20.34	.06	.10
White Minorcas	32	7.47			20.40		
Buff Orpingtons	36	9.19	.03	.02	22.44	3.16	8.66
Barred Plymouth Rocks	36	9.22			19.28		
Buff Orpingtons	36	9.19	1.72	1.30	22.44	2.04	4.92
White Minorcas	32	7.47			20.40		
Barred Plymouth Rocks	36	9.22	1.75	1.38	19.28	1.12	2.58
White Minorcas	32	7.47			20.40		

GROUP B, EXAMINED AT 40 DAYS							
Rhode Island Reds	51	3.61	1.17	1.76	14.46	1.09	1.20
White Plymouth Rocks	55	2.44			13.37		
Rhode Island Reds	51	3.61	.19	.28	14.46	2.92	5.24
Buff Orpingtons	50	3.42			17.38		
Rhode Island Reds	51	3.61	.10	.15	14.46	2.32	4.50
Barred Plymouth Rocks	48	3.71			16.78		
Rhode Island Reds	51	3.61	4.39	4.98	14.46	5.85	13.42
White Minorcas	40	8.00			20.31		
White Plymouth Rocks	55	2.44	.98	1.75	13.37	4.01	4.33
Buff Orpingtons	50	3.42			17.38		
White Plymouth Rocks	55	2.44	1.27	2.34	13.37	3.41	3.78
Barred Plymouth Rocks	48	3.71			16.78		
White Plymouth Rocks	55	2.44	5.56	7.09	13.37	6.94	8.08
White Minorcas	40	8.00			20.31		
Buff Orpingtons	50	3.42	.29	.47	17.38	.60	1.09
Barred Plymouth Rocks	48	3.71			16.78		
Buff Orpingtons	50	3.42	4.58	5.47	17.38	2.93	6.12
White Minorcas	40	8.00			20.31		
Barred Plymouth Rocks	48	3.71	4.29	5.20	16.78	3.53	8.09
White Minorcas	40	8.00			20.31		

⁴ The White Minorca chicks all came from the same flock, but those of group D were sired by different and somewhat smaller White Minorca cocks.

TABLE 2.—The comparative resistance to nematodes of 3 breeds and 2 varieties of a fourth breed of chickens as determined by the number and length of the *Ascaridia lineata* recovered from each breed at autopsy—Continued

GROUP C, EXAMINED AT 51 DAYS

Breed	Hosts	Worms	Difference	Ratio, D/E	Mean length of worms	Difference	Ratio
	Number	Mean number			Mm.		
Rhode Island Reds.....	43	2.84	0.55	0.98	20.18	0.28	0.52
White Plymouth Rocks.....	61	3.39			20.46		
Rhode Island Reds.....	43	2.84	2.41	2.95	20.18	3.74	7.06
Buff Orpingtons.....	53	5.25			23.92		
Rhode Island Reds.....	43	2.84	.13	.27	20.18	.54	1.17
Barred Plymouth Rocks.....	52	2.71			19.64		
Rhode Island Reds.....	43	2.84	.33	.72	20.18	1.15	1.99
White Minorcas.....	49	2.51			19.03		
White Plymouth Rocks.....	61	3.39	1.86	2.23	20.46	3.46	7.99
Buff Orpingtons.....	53	5.25			23.92		
White Plymouth Rocks.....	61	3.39	.68	1.32	20.46	.82	2.37
Barred Plymouth Rocks.....	52	2.71			19.64		
White Plymouth Rocks.....	61	3.39	.88	1.79	20.46	1.43	2.91
White Minorcas.....	49	2.51			19.03		
Buff Orpingtons.....	53	5.25	2.54	3.24	23.92	4.28	12.06
Barred Plymouth Rocks.....	52	2.71			19.64		
Buff Orpingtons.....	53	5.25	2.74	3.55	23.92	4.89	10.08
White Minorcas.....	49	2.51			19.03		
Barred Plymouth Rocks.....	52	2.71	.20	.50	19.64	.61	1.49
White Minorcas.....	49	2.51			19.03		

GROUP D, EXAMINED AT 54 DAYS

Rhode Island Reds.....	59	1.58	0.43	1.38	16.59	0.34	0.47
White Plymouth Rocks.....	61	1.15			16.93		
Rhode Island Reds.....	59	1.58	.13	.36	16.59	1.35	1.91
Buff Orpingtons.....	52	1.71			17.94		
Rhode Island Reds.....	59	1.58	.31	.99	16.59	1.33	1.90
Barred Plymouth Rocks.....	51	1.27			17.92		
Rhode Island Reds.....	59	1.58	.23	.60	16.59	7.15	9.52
White Minorcas.....	26	1.35			23.74		
White Plymouth Rocks.....	61	1.15	.56	1.80	16.93	1.01	1.26
Buff Orpingtons.....	52	1.71			17.94		
White Plymouth Rocks.....	61	1.15	.12	.48	16.93	.99	1.25
Barred Plymouth Rocks.....	51	1.27			17.92		
White Plymouth Rocks.....	61	1.15	.20	.60	16.93	6.81	8.12
White Minorcas.....	26	1.35			23.74		
Buff Orpingtons.....	52	1.71	.44	1.41	17.94	.02	.03
Barred Plymouth Rocks.....	51	1.27			17.92		
Buff Orpingtons.....	52	1.71	.36	.94	17.94	5.80	7.02
White Minorcas.....	26	1.35			23.74		
Barred Plymouth Rocks.....	51	1.27	.08	.24	17.92	5.82	7.11
White Minorcas.....	26	1.35			23.74		

GROUP E, EXAMINED AT 65 DAYS

Rhode Island Reds.....	36	1.36	0.34	0.83	17.58	2.34	2.52
White Plymouth Rocks.....	37	1.70			19.92		
Rhode Island Reds.....	36	1.36	.36	.84	17.58	1.70	1.40
Buff Orpingtons.....	21	1.00			15.88		
Rhode Island Reds.....	36	1.36	.67	1.93	17.58	.16	.13
Barred Plymouth Rocks.....	35	.69			17.58		
Rhode Island Reds.....	36	1.36	.17	.46	17.58	.13	.14
White Minorcas.....	43	1.19			17.71		
White Plymouth Rocks.....	37	1.70	.70	1.91	19.92	4.04	4.14
Buff Orpingtons.....	21	1.00			15.88		
White Plymouth Rocks.....	37	1.70	1.01	3.75	19.92	2.50	2.47
Barred Plymouth Rocks.....	35	.69			17.42		
White Plymouth Rocks.....	37	1.70	.51	1.75	19.92	2.21	3.57
White Minorcas.....	43	1.19			17.71		
Buff Orpingtons.....	21	1.00	.31	.95	15.88	1.54	1.21
Barred Plymouth Rocks.....	35	.69			17.42		
Buff Orpingtons.....	21	1.00	.19	.59	15.88	1.83	1.83
White Minorcas.....	43	1.19			17.71		
Barred Plymouth Rocks.....	35	.69	.50	2.49	17.42	.29	.28
White Minorcas.....	43	1.19			17.71		

In reading across table 2, it will be seen that when the Rhode Island Reds are compared with the White Plymouth Rocks, the former have fewer or shorter worms in 7 of the 10 comparisons. Of these 7, only 1 is significant, indicating that no marked difference in the resistance occurred between these two breeds at the different ages.

By comparing the Rhode Island Reds successively through the various groups with the White Plymouth Rocks, Buff Orpingtons, Barred Plymouth Rocks, White Minorcas, and finally with the White Leghorns (table 1), it may be seen that in 34 of the 50 comparisons, the Rhode Island Reds had fewer or shorter *Ascaridia lineata* than did the other breeds with which they were compared. Of these 34 cases, 21 were significant (table 3).

In comparing the average number and length of the *Ascaridia lineata* from the White Plymouth Rocks with those from the Buff Orpingtons (table 2), it is seen that in eight instances fewer or shorter *Ascaridia lineata* were found in the White Plymouth Rocks. Three of these cases represented significant differences. By making similar comparisons of the White Plymouth Rocks with the Barred Plymouth Rocks, White Minorcas, Rhode Island Reds, and with the White Leghorns (table 1) it was found that in 32 of 50 comparisons the White Rocks had fewer or shorter worms. Twelve of these differences were significant, as compared with 21 for the Rhode Island Reds (table 3).

TABLE 3.—Summary of breed resistance to *Ascaridia lineata*, in analysis of data from tables 1 and 2

Breed	Fifty comparisons of the number or length of <i>A. lineata</i> for each breed		
	Hosts	Total cases of fewer or shorter <i>A. lineata</i> than in the other breeds or varieties	Cases of significantly fewer or shorter <i>A. lineata</i>
	Number	Number	Number
Rhode Island Reds	224	34	21
White Plymouth Rocks	250	32	12
Barred Plymouth Rocks	222	32	13
White Minorcas	190	22	8
Buff Orpingtons	212	19	6
White Leghorns	253	11	2

It was interesting to note that when the average number and length of *Ascaridia lineata* from the Barred Plymouth Rocks were compared with these data for the other breeds, there was exactly the same number of cases, 32, as for the White Plymouth Rocks, in which the number of the worms was smaller or their length shorter than in the other breeds. The number of significant cases, 13, was approximately the same as that for the White Plymouth Rocks (table 3).

Fourth in the list of most resistant breeds were the White Minorcas. When the *Ascaridia lineata* from them were compared with those from the other breeds and varieties, it was found that in 22 of the 50 comparisons, the White Minorcas averaged fewer or smaller nematodes. Of this number 8 cases were considered significant (table 3).

A further examination of tables 1 and 2 shows that the Buff Orpingtons, while being perhaps the heaviest breed studied, were far from being the most resistant to the parasites. Of 50 comparisons with other breeds, only 19 showed fewer or smaller parasites, and of this number only 6 were significant. It was thought that the Buff Orpingtons in group C may have been chilled and that therefore their resistance might have been affected, but another test (group A, table 2) showed that the Buff Orpingtons were quite susceptible to *Ascaridia lineata* both as to number and length of worms.

The standing of the White Leghorns in the list has been discussed in a previous section. In table 1 it is seen that comparatively large numbers of rather long worms from the White Leghorns occur throughout; and table 3 shows that in only 11 of 50 comparisons were the worms from the White Leghorns fewer or shorter than those from the other breeds; furthermore, in only 2 of these cases were the differences significant.

From these tests on 1,351 chickens, each of which was fed 50 ± 5 embryonated eggs of the nematode *Ascaridia lineata*, the authors conclude that the White Leghorns were the least resistant and that the Rhode Island Reds were the most resistant to the worms, the others in order of their degree of resistance being the White Plymouth Rocks and Barred Plymouth Rocks about equally resistant, the White Minorcas, and the Buff Orpingtons. No explanation of these differences is available other than the possibility of the Leghorns' greater utilization of nervous energy resulting in reduced resistance to the worms; or that the White Leghorns being the oldest of these breeds or varieties may have been the normal or more tolerant host of this worm and hence less resistant to it.

AGE RESISTANCE

By arranging according to age the different groups of each breed or variety of chickens and giving the corresponding average numbers and lengths of *Ascaridia lineata*, comparisons are readily made for evidence of age resistance of the chickens to the parasites. It will be noted (table 4) that the breeds in group A, the youngest birds, were examined at 33 days of age and those of group E, the oldest, at 65 days. On examining the average numbers of worms from the White Leghorns it is seen that there was a fairly gradual reduction in numbers of worms from group to group, indicating the development of age resistance as the birds grew older. More or less similar results on numbers of worms occurred for the other breeds or varieties as shown in table 4. Some of these differences are significant. Others, doubtless, would have been with larger numbers of hosts.

The average length of worms of the White leghorns in group A was 22.08 mm and that of those in group B, 18.19 mm, a difference of 3.89 mm, which was significant (table 4). Similar comparisons between the Rhode Island Reds, Buff Orpingtons, White Plymouth Rocks, and Barred Plymouth Rocks in groups A and B are significant. While there are irregularities in the rates of growth, and longer worms in the older groups occur, the evidence points to the development of an age resistance to the growth of these worms. In the Buff Orpingtons the average length of worms decreased 6.56 mm in 1 month, the worms averaging 22.44 mm from chickens 33 days old and 15.88

mm from birds 65 days old. In all cases the worms from the chickens 65 days of age are shorter than those from chickens 33 days old (table 4).

TABLE 4.—*Development of age resistance to Ascaridia lineata in certain breeds and varieties of chickens*

Breed and group	Age when examined	Average of <i>A. lineata</i>	Average length of <i>A. lineata</i>	Breed and group	Age when examined	Average of <i>A. lineata</i>	Average length of <i>A. lineata</i>
White Leghorns.	Days	Number	Mm	White Plymouth Rocks:	Days	Number	Mm
A	33	8.28	22.08	A	33	7.08	20.34
B	40	7.34	18.19	B	40	2.44	13.37
C	51	5.73	23.83	C	51	3.39	20.46
D	54	1.22	20.00	D	54	1.15	16.93
E	65	2.00	21.45	E	65	1.70	19.92
Rhode Island Reds:				Barred Plymouth Rocks:			
A	33	4.89	17.66	A	33	9.22	19.28
B	40	3.61	14.46	B	40	3.71	16.78
C	51	2.84	20.18	C	51	2.71	19.64
D	54	1.58	16.50	D	54	1.27	17.92
E	65	1.36	17.58	E	65	.69	17.42
Buff Orpingtons:				White Minorcas:			
A	33	9.19	22.44	A	33	7.47	20.40
B	40	3.42	19.38	B	40	8.00	20.31
C	51	5.25	23.92	C	51	2.51	19.03
D	54	1.71	17.94	D	54	1.35	23.74
E	65	1.00	15.88	E	65	1.19	17.71

Attention should be called to the average length of the *Ascaridia lineata* from the various breeds in group C. Although these chickens are 11 and 18 days older than those in groups B and A respectively, the worms average slightly longer in each case, except in the White Minorcas. Whether this resulted from a more vigorous strain of *A. lineata* or from more susceptible groups of chickens is uncertain. In number of worms, the chickens in group C are relatively high. Thus the Leghorns in group C averaged 5.73 worms, and those in group D, only 3 days older, 1.22 worms. Similarly, the Buff Orpingtons in group C average 5.25 worms, and those in group D 1.71 worms. A similar comparison may be made for the White Plymouth Rocks. A plausible explanation is that in the group-C series of experiments a more vigorous strain of *A. lineata* was utilized (table 4).

DISCUSSION

Experimental evidence is available for showing marked differences in the resistance of different species of animals to the same parasite. The Brahman cattle (*Bos indicus*), as is well known, are able completely to escape parasitism with the cattle tick organism, *Babesia bigemina*, which is very pathogenic to domestic cattle (*Bos Taurus*). Swine are not susceptible to the human ascarid, which is morphologically indistinguishable from that of the pig (25). Ackert and Grant (8) found that the dog tapeworm *Taenia pisiformis* would grow in kittens but that the specimens were markedly reduced in size in the feline hosts; and Scott (30) infected cats with *Ancltyostoma caninum* larvae from a dog infestation of this hookworm and found that the worms grew slower and reached a smaller final size in the cats than did similar worms in the dogs.

The present studies, however, including a preliminary report by Ackert, Eisenbrandt, Glading, and Wilmoth (6), appear to be the

first to give evidence of different degrees of host resistance between breeds of the same species to helminthic infection.

Breed resistance to bacteria was demonstrated by Lambert (24), who infected four breeds of chickens with *Salmonella gallinarum*, producing fowl typhoid. After giving all the same-sized dose, he compared the number of deaths. The lowest mortality occurred in the White Plymouth Rocks; next in order were the White Leghorns, White Wyandottes, and the Rhode Island Reds. The only similarity of Lambert's results to those from the present experiments was that the White Plymouth Rocks ranked high among the resistant fowls in each case. In his work the White Leghorns had the second smallest number of deaths, whereas in the present experiments the White Leghorns were the least resistant to *Ascaridia lineata*. His results, however, were based on percentages of deaths, and these on the degree of resistance to parasites.

Other data on the comparative mortality of breeds of chickens are available in the work of Harris and Boughton (19), who concluded that the White Wyandottes had a significantly higher death rate during the first laying year than Rhode Island Reds or White Leghorns. These authors inferred that the death rate was somewhat higher in Rhode Island Reds than in White Leghorns.

Dudley (17), after a careful study of available data, including that of Harris and Boughton, concluded that there were no essential differences in the death rates of these breeds.

Similar results were obtained by Weaver (34), who studied the comparative mortality (due to various causes including parasitism) of White Leghorns and Barred Plymouth Rocks and found no constant differences between the breeds.

Judging from these results, there appear to be no marked differences in mortality in breeds of chickens and little if any relationship between death rates and the degree of resistance of breeds or varieties of chickens to parasitism. In this connection it is interesting to note that while the White Leghorns were the least resistant to the parasites, their death rate in the pens was markedly lower than that of any other breed.

The analysis of the data on the resistance of the White Minorcas to the growth of the *Ascaridia lineata* pointed to the possibility of two strains of White Minorcas. The White Minorcas in groups C and E (table 1) were more resistant than the White Leghorns, but the Minorcas in groups B and D were more susceptible. The Minorcas in groups C and E were secured in 1932-33, whereas those in groups B and D were secured in 1933-34. In both years the chickens came from the same flock; but a change was made in cockerels in the second-year flock. When the growth rates of the White Minorcas in the 2 years were compared, it was found that the White Minorcas used the first year were decidedly heavier than those used the second year, indicating two strains of this breed. They, at least, were of different genetical constitution. Strains of chickens varying in resistance to disease have been found by Roberts (28), who secured a 41 percent survival of chickens (infected with *Bacterium pullorum*) from selected stock as compared with a 29 percent survival from random chicks. Lambert (24) found slight differences in the degrees of resistance of two strains of White Leghorns to *Salmonella gallinarum* the causative organism of fowl typhoid.

In regard to evidence of age resistance of chickens to *Ascaridia lineata* (table 4) it was found that the worms from the group C chickens were much longer than those from the group B chickens, which were younger. This finding is evidence of a more vigorous strain of *A. lineata* in the group C fowls. Different strains of helminths were detected by Scott (31), who found dog hookworms (*Ancylostoma caninum*) that were obtained from a cat infestation to be exceptionally adapted to the cat but almost wholly unable to live in puppies. Scott likewise obtained a dog strain of this parasite that was very viable in puppies but not in kittens.

Explanations of the difference in resistance among the breeds, varieties and strains of chickens to *Ascaridia lineata* are not readily available. For a general review of animal resistance to helminths the reader is referred to Chandler (16). A recent review in which immunity to protozoan parasites is stressed has been given by Taliaferro (32). Concerning size as a factor, the heavier breeds and varieties: Rhode Island Reds, White Plymouth Rocks, and Barred Plymouth Rocks, were more resistant to the *A. lineata* than the lighter Leghorns and White Minorcas; but the heavy Buff Orpingtons proved to be slightly more susceptible than the White Minorcas. Further evidence of heavier birds being more resistant is afforded by the 1932-33 White Minorcas, which were both heavier and more resistant than were the lighter 1933-34 Minorcas. In general behavior the heavy breeds were comparatively inactive, while the lighter, more susceptible Leghorns and Minorcas were markedly more active and nervous. It is conceivable that this greater utilization of energy might be unfavorable to the development of inhibiting factors.

A possible explanation of the results might be that the chickens tested represented susceptible and resistant strains within the breeds and varieties; but the chickens compared came from more than one flock in all cases except those of the White Minorcas and the White Plymouth Rocks. So, it would appear that these tests were made upon a fair section of fowl population.

It is possible that the theory of the abnormal host postulated by Ransom (27) and elaborated by Sandground (29) is applicable. On this basis the White Leghorns, which are the most susceptible to the *Ascaridia lineata*, would be considered the normal hosts and the heavy breeds, abnormal or more resistant hosts. The fact that the rather susceptible White Minorcas are a Mediterranean breed and therefore closely related to the White Leghorns lends support to this hypothesis. Furthermore, Ackert and Eisenbrandt (5) found White Leghorn chickens to be more susceptible to *A. lineata* than were Bronze turkeys, which likewise might be considered abnormal hosts, especially since they were parasitized with worm eggs from a chicken infestation. But even this does not indicate how the resistance works. As previous studies by Ackert (1) and by Porter and Ackert (26) have shown that blood loss reduces the resistance of chickens to *A. lineata*, the writers favor the view that resistance is an inhibiting complex which so operates as to retard the growth and development of these intestinal worms. Whether the inhibitors are in the nature of macrophages which may attack the worms when they are in close proximity to the intestinal epithelium, whether the worm devours a host substance that checks its growth, or whether the resistance is due to other factors, is uncertain.

SUMMARY AND CONCLUSIONS

Evidence is presented for the first time on different degrees of resistance among breeds and varieties of one species to the same helminth. The data supporting the conclusions were derived from experiments on 1,351 chickens of 4 breeds and 2 varieties of a fifth breed given the same numbers of eggs from the nematode *Ascaridia lineata* (Schneider). The criteria for judging the resistance were the average number and length of the *Ascaridia lineata* from each group of chickens under comparison.

Most resistant to the parasites were the heavy breeds and varieties: Rhode Island Reds, White Plymouth Rocks, and Barred Plymouth Rocks; the most susceptible were the White Leghorns, Buff Orpingtons, and White Minorcas. A strain of heavy White Minorcas proved to be more resistant to the *Ascaridia lineata* than a lighter strain of the same breed with different genetic constitution. Factors in the differences in resistance appear to include greater utilization of nervous energy by the most susceptible breed, possible differences in strains within a breed, and the normality or tolerance of the host breeds.

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INHERITANCE OF COLD RESISTANCE IN WINTER WHEAT, WITH PRELIMINARY STUDIES ON THE TECHNIC OF ARTIFICIAL FREEZING TESTS¹

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INTRODUCTION

When this study of the inheritance of cold resistance in winter wheat was initiated in 1929, it was discovered that few data were available to show the exact procedure which might be followed in conducting artificial freezing tests. While a number of studies had been reported, there were some phases of the technic which had not been fully investigated, and others about which there was much disagreement. Consequently a series of experiments were set up in order to develop methods that would be suitable for the problem at hand. The first part of this paper deals with these experiments in technic, and the second part with the inheritance of cold resistance in winter wheat.

REVIEW OF LITERATURE

The effect of low temperature on plant tissue has been the subject of study for many years. The work of previous investigators has been thoroughly reviewed by many authors, including Harvey (6),³ Newton (11), Åkerman (2), Martin (9), Salmon (18); consequently, only a few of the more-pertinent papers will be taken up here.

Hill and Salmon (8) and Salmon (18) obtained high correlations between the results of artificial freezing of varieties of wheat and the field survival as determined in the uniform winter hardiness nurseries.

Åkerman (2, 3), Maximov (10), and Quisenberry (15) found that results obtained by direct freezing methods agreed very well with those obtained in field trials.

Martin (9) concluded that "freezing under controlled temperatures offers the greatest promise in measuring the hardiness of wheat plants by laboratory methods."

Of recent contributions on the inheritance of winter hardiness, the following are of special interest.

Nilsson-Ehle (12) and Åkerman (1) crossed two varieties of intermediate hardiness. They obtained some lines that were less hardy and others that were more hardy than the parents, and concluded that winter resistance is a quantitative character, controlled by several Mendelian factors.

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³ Reference is made by number (italic) to Literature Cited, p. 634.

Gaines, according to Schafer (19), reported that winter hardiness reacted as a recessive, since a majority of the F_3 rows showed severe winter injury.

Hayes and Aamodt (7), Quisenberry and Clark (16), and Quisenberry (15), working with different wheat crosses, concluded that winter hardiness is a heritable character and is probably controlled by many genetic factors.

MATERIALS AND METHODS

In all tests dealing with technic, three varieties of wheat having a wide range in winter hardiness were used. These varieties were, in order of ascending hardiness, Poole, Michikof, and Minhardi.

For inheritance studies of cold resistance, crosses between pure lines of Poole and Minhardi were made in 1930. The plants in the F_2 generation were subjected to artificial freezing tests during the winter of 1932-33, and those of the F_3 generation during 1933-34. Because of the unreliability of survival readings when based on single plants, the cold resistance of the individual F_2 plants could not be used as a basis on which to make a satisfactory estimate. Consequently 75 seedlings, grown from the seed of each plant, were subjected to the standard freezing test, and the average estimated survival of the seedlings was used as a basis for determining the cold resistance of each F_2 plant, taken at random. Several F_2 plants that showed wide differences in respect to relative cold resistance, were further tested in the F_3 generation. Each test included the seedlings from 10 F_2 or F_3 plants as well as Poole and Minhardi.

All results herein reported were obtained from wheat seedlings grown, hardened, frozen, and thawed in the greenhouse. The freezing was accomplished in a cold chamber by means of the F_{12} (dichlorodifluoro-methane), direct-expansion refrigeration machine shown in figure 1. The chamber is a well-insulated compartment with inside dimensions of 10 by 4 by 3 feet. The temperature was regulated within a range of $\pm 1^\circ$ F. by a thermometer controller. By means of a recording thermometer the exact temperatures during the course of each test were recorded.

Four-inch clay pots were filled with Crosby silt loam soil, and in each pot 7 seeds were placed to insure a uniform stand of 5 plants per pot. Only wheat seedlings that were grown in the greenhouse during December, January, February, and March were used. The temperature in the greenhouse was maintained at about 60° F., until the seedlings were about 5 weeks old. Eight hours before exposure to the freezing chamber, the soil was saturated with water. At 5 p. m., 180 pots containing the seedlings were placed in the chamber and hardened at $+34^\circ$ for a period of 15 hours. Artificial light from two 200-watt bulbs was used during hardening. At 8 a. m., the next morning, the lights in the chamber were turned out, and the thermometer controller set at $+22^\circ$ for freezing. Within a half hour the temperature in the chamber was 22° and remained until 4 p. m.

Immediately after freezing, the seedlings were transferred to the greenhouse, held at a temperature of about 60° F., and kept well

watered in an attempt to revive them. Seven days after freezing records were taken of the average estimated percentage of survival. The method reported by Quisenberry (15) was used in estimating the average survival. This method takes into account not only the number of living and dead plants, but also the degree of injury to surviving plants.

EXPERIMENTS IN TECHNIC

The experiments dealing with technic were conducted over a period of three winters, during which time more than 85,000 seedlings were frozen. The following steps in procedure were studied: (1) Freezing temperatures, (2) hardening conditions, (3) age of wheat seedlings at freezing, (4) moisture content of the soil, and (5) treatment of plants after exposure to freezing. A large number of preliminary

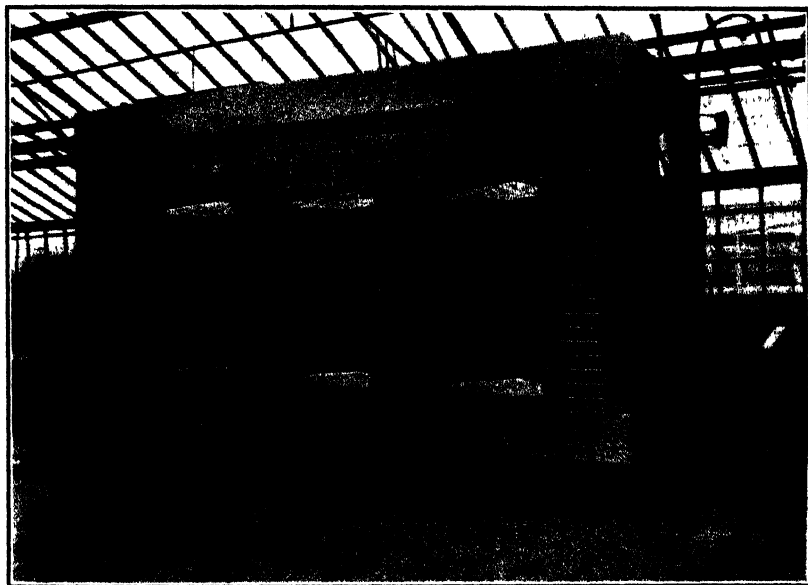


FIGURE 1.- Chamber and compressor used for controlled freezing tests.

experiments were conducted in which various temperatures and duration of exposures were tested in order to obtain information for carrying out the further tests which formed the basis for establishing a standard freezing test. Only the data from these later experiments are reported.

FREEZING TEMPERATURES

From preliminary trials, it was found that the temperature range in which different degrees of injury would result was rather narrow. After this range had been determined a number of tests were conducted to ascertain the freezing temperatures which would express the greatest differential injury between the varieties studied. The data obtained with the three varieties of wheat are shown in table 1.

TABLE 1.—*Influence of freezing temperature on the estimated survival of wheat plants of 3 varieties*¹

Plants (number)	Freezing conditions		Average estimated survival ²		
	Temperature	Period	Poole	Michikof	Minhardi
	° F.	Hours	Percent	Percent	Percent
900.....	18	8	8.1	12.8	22.2
900.....	20	8	5.1	18.3	28.9
2,700.....	22	8	21.9	35.3	54.4
900.....	26	4	27.5	39.0	53.7
	18	4			

¹ Artificial lights were used during hardening, which consisted in exposure to a temperature of 34° F. for 15 hours.

² The odds are 19 : 1 for the following differences: 2.95 when comparing 2 means of 900 plants; 2.62 when comparing a mean of 900 with a mean of 1,800; 2.41 when comparing a mean of 900 with a mean of 2,700; 2.27 when comparing 2 means of 1,800; 2.01 when comparing a mean of 1,800 with a mean of 2,700; and 1.71 when comparing 2 means of 2,700.

It will be noted that as the freezing temperatures were increased to 22° F., the estimated average survival also increased. Plants frozen gradually, that is, the first 4 hours at 26° and the second 4 hours at 18°, showed about the same survival as those frozen at 22° for 8 hours. As a result of these tests, a freezing temperature of 22° for a period of 8 hours was adopted as the standard.

HARDENING CONDITIONS

To determine a suitable hardening treatment by which the greatest differential injury could be obtained, wheat plants were subjected to various hardening conditions, as shown in table 2.

An inspection of table 2 shows that wheat plants which were not hardened before being subjected to a freezing treatment of 22° F. for 8 hours showed a much lower survival than those previously hardened at various temperatures for 15 to 39 hours and subjected to the same freezing treatment. Plants hardened at 34° for 15 hours showed the greatest difference in estimated survival between the three varieties representing nonhardy, mid-hardy, and hardy types. Under the conditions of these experiments, it appears that plants hardened for a longer period than 15 hours at a temperature of 34° or higher show a higher estimated survival; however, the differences between nonhardy and hardy varieties are smaller. Therefore a 15-hour exposure at 34° was used as a hardening period. It may be that this period will not prove best under all conditions; for these conditions, however, it serves as a basis for standardizing a suitable technic by which relative cold resistance of wheat seedlings may be determined.

TABLE 2.—*Influence of length of exposure at various temperatures in the hardening chamber on the estimated survival of wheat plants of 3 varieties to artificial freezing at 22° F. for 8 hours*

Plants (number)	Hardening conditions ¹		Average estimated survival ²		
	Temperature	Period	Poole	Michikof	Minhardi
	° F.	Hours	Percent	Percent	Percent
900.....	(3)	(3)	12.7	25.5	28.1
2,700.....	34	15	21.9	35.3	54.4
1,800.....	40	15			
	34	24	40.5	53.3	60.6
1,800.....	34	39	30.2	38.8	49.6
1,800.....	36	39	32.5	42.3	51.0

¹ Artificial lights were used during hardening.

² See footnote 2 to table 1 for difference necessary for significance.

³ No hardening; control.

AGE OF WHEAT SEEDLINGS AT FREEZING

To determine the age at which wheat seedlings could be most satisfactorily subjected to comparative cold resistance tests, seedlings of three varieties of wheat ranging in age from 14 to 46 days were grown in the greenhouse and subjected to freezing tests. The estimated average survival of the seedlings is shown in table 3.

TABLE 3.—*Influence of age on the cold resistance of groups of 900 wheat seedlings of different ages and of 3 varieties*¹

Age of seedlings (days)	Average estimated survival ²			Age of seedlings (days)	Average estimated survival ²		
	Poole	Michikof	Minhardi		Poole	Michikof	Minhardi
	Percent	Percent	Percent		Percent	Percent	Percent
14.....	0 0	1.0	3 1	32.....	23.0	32.5	47.7
21.....	10 3	13 6	20 8	39.....	23.0	34.6	46.3
24.....	11.4	22.4	32 4	43.....	21.0	33.5	50.7

¹ All plants were hardened at 34° F. for 15 hours and frozen at 22° for 8 hours. Artificial lights were used during hardening.

² See footnote 2 to table 1 for difference necessary for significance.

The data show that seedlings up to the age of 32 days were quite susceptible to cold. After that period, however, there did not appear to be any difference in cold susceptibility up to 46 days. In making comparative tests for cold resistance, therefore, wheat plants were grown in the greenhouse for about 5 weeks prior to the standard freezing test.

MOISTURE CONTENT OF THE SOIL

There is much disagreement among investigators as to whether plants show a greater injury in dry or wet soil following natural or artificial freezing.

Salmon (17) reports a lower survival of barley and oats in dry soil than in medium wet or wet soil during the first season; however, directly contrary results were obtained the second season. Later, Salmon (18) found in some tests a greater injury in dry soil than in wet; however, in other tests, where the various lots were frozen slowly, no differences in injury were observed. Sellschop and Salmon (20) state that "cowpeas, peanuts, maize, and velvet beans were far more severely injured in wet than in dry soil." Peltier and Tysdal (14) and Tysdal (21) concluded that plants in dry soil were injured more than those in wet soil.

In experiments conducted by the writer, wheat plants were grown in 4-inch pots, containing Crosby silt loam soil, until 5 weeks of age. Just prior to the freezing test the soil in all of the pots was allowed to dry for about 2 days until it contained 12.4 percent moisture. Eight hours before exposure to the freezing-chamber tests, one-half of the pots were saturated with water and contained 23.5 percent moisture, while the other remained dry. Both lots were then placed in the chamber and subjected to the standard freezing test. The average estimated survival of the three varieties from four tests is shown in table 4.

TABLE 4.—*Effect of moisture content of soil on the estimated survival of groups of 900 plants of 3 varieties of winter wheat by freezing*

Variety	Average estimated survival in ¹ —	
	Dry soil	Wet soil
	Percent	Percent
Poole.....	30.5	23.0
Michikof.....	53.6	37.4
Minhardi.....	65.0	48.5

¹ See footnote 2 to table 1 for difference necessary for significance.

The data show that, under the conditions of these experiments, winter wheat plants revealed a greater estimated survival in dry than in wet soil. Soil temperatures taken during the course of a freezing test indicated that the temperature fell more rapidly in dry soil than in wet. Since variations in moisture content of the soil produce marked differences in survival, in all comparative hardiness tests all lots were thoroughly watered 8 hours before a test. Natural drainage left the soil in the different pots at approximately the same moisture content.

TREATMENT OF PLANTS AFTER EXPOSURE TO FREEZING

Anderson and Kiesselbach (4), Åkerman (2), and Peltier and Tysdal (13, 14) found that slow thawing increased the percentage of survival in plants; Carrick (5) and Weimer (22) found that slow thawing did not increase the percentage of survival.

To determine the influence of slow and rapid thawing on injury to plants, experiments were conducted in which one-half of the pots, after a regular freezing test, were taken immediately from the chamber to the greenhouse maintained at 60° F., while the other half of the pots were left in the chamber at 34° for 15 hours before they were placed in the greenhouse. The results obtained are shown in table 5.

TABLE 5.—*Influence of the rate of thawing on the estimated survival of groups of 900 wheat plants of 3 varieties ¹*

Variety	Average estimated survival ²	
	Slow thawing	Rapid thawing
	Percent	Percent
Poole.....	8.9	8.4
Michikof.....	25.4	26.4
Minhardi.....	44.5	47.3

¹ Data are not comparable with those in other tables since plants were grown in shaded portion of greenhouse.

² See footnote 2 to table 1 for difference necessary for significance.

TABLE 6.—Estimated survival of parents, and F_1 , F_2 , and F_3 generations of Poole and Minhardi cross, as determined by controlled freezing tests during 1932-33 and 1933-34

Parent or cross	Generation	Year	Survival of F_1	Estimated survival for indicated class centers												Lines	Mean	C. V.
				7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5			
				Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent	Per- cent			
P ₁																		
Do.		1933	Percent															
Minhardi		1933																
Do.		1934																
F_1		1934																
F_2		1933																
3-2		1934	5.5	1	3	10	31	56	82	30	3	2						
3-1		1934	14.6	8	11	9												
3-3		1934	19.0	2	7	16	3											
3-4		1934	27.7	1	2	5	7	6	1									
6-55		1934	37.7			2		8	23	6								
3-6		1934	40.3															
7-9		1934	48.8															
3-13		1934	54.1															
		1934																

1 Average of 34 F_1 plants.

The data in these tests show no significant differences in the estimated survival between slow and rapid thawing of wheat plants. Consequently, in all regular tests, the plants were removed directly from the chamber to the greenhouse.

INHERITANCE OF COLD RESISTANCE IN WINTER WHEAT

In studying the inheritance of cold resistance in soft winter wheat, F_1 , F_2 , and F_3 generations, together with their parents, were grown in the greenhouse and subjected to artificially controlled freezing tests during the winters of 1932-33 and 1933-34. In this period over 40,600 seedlings were frozen and the cold resistance of each determined. The data obtained are arranged in frequency distributions and shown in table 6.

The results show that the nonhardy parent, Poole, had an estimated survival of 24.1 and 23.3 percent, while the hardy parent, Minhardi, had an estimated percentage survival of 53 and 51.5 for 1932-33 and 1933-34, respectively. Since the coefficients of variability for the parents are small, one might conclude that both parents, though widely different, are quite homozygous for cold resistance. The average estimated survival of 34 F_1 plants tested was 36.5 percent. The estimated survival of the F_2 plants varied from 7.5 to 52.5 percent with an average survival of 34.6 percent; that is, the F_2 plants were about intermediate to the parents in cold resistance. Some lines appeared more susceptible than Poole while others seemed as resistant as Minhardi; but the majority fell into the intermediate classes.

TABLE 7.—Mean of the estimated survival of several lines in comparison with the survival of the nonhardy or hardy parent

Parent or cross	Mean survival	Difference between progeny and similar parent	Dif./S. E.
Poole.....	23.34±0.503	—	—
3-2.....	17.65±.735	5.69±0.926	6.14
3-1.....	21.43±.637	1.91±.850	2.25
7-9.....	46.59±.452	4.87±.852	5.72
3-13.....	46.74±1.166	4.72±1.363	3.46
Minhardi.....	51.46±.722	—	—

The data show that the survival found in the F_2 plants agreed very well with that found in the F_3 ; that is, lines that showed susceptibility to frost injury in the F_2 also were tender in the F_3 , while other lines that proved to be cold-resistant in the F_2 were also relatively cold-resistant in the F_3 . Some F_2 families appeared to be even tenderer than the nonhardy parent, while others were almost as hardy as Minhardi. The extent to which transgressive segregation appeared and parental types were recovered is shown in table 7.

An examination of these data reveals that strains 3-2 and 3-1, when compared with Poole, show a mean difference of 5.69 and 1.91, respectively. The fact that these differences are 6.14 and 2.25 times the standard error shows that they are significant. This indicates that both strains are less winter-hardy than the nonhardy parent, and strongly suggests transgressive segregation in the direction of

nonwinter hardiness. The two most winter-hardy of the segregates studied (7-9 and 3-13) are less hardy than Minhardi, the hardy parent.

The great increase in the variability between the parents and the F_2 generation, the reappearance of the nonhardy parent, and the recombination of strains less winter-hardy than the nonhardy parent (transgressive segregation) indicate clearly the segregation of genetic factors. The results indicate, therefore, that cold resistance is inherited in the same manner as other quantitative characters; however, the number of genetic factors involved cannot be determined from the data. These results confirm the findings of other investigators, especially those of Nilsson-Ehle (12), Hayes and Aamodt (7), and Quisenberry (15).

SUMMARY

Studies on the technic of control hardiness tests and the inheritance of cold resistance in winter wheat varieties were made on wheat seedlings grown in the greenhouse during the winters of 1931-32, 1932-33, and 1933-34.

In experiments dealing with the development of a suitable technic for measuring cold resistance, over 85,000 wheat seedlings were frozen and their survival determined.

The steps involved in developing a suitable method for determining cold resistance in wheat seedlings under controlled conditions are presented.

Wheat plants hardened for 15 hours at 34° F., under artificial illumination, show wider differences in estimated survival between varieties representing nonhardy, midhardy, and hardy types, than when hardened for a longer period at the same or different temperatures.

Wheat seedlings up to the age of 32 days were much more susceptible to cold than older plants. Wheat plants 32, 39, and 46 days old showed approximately the same survival when subjected to the standard artificial freezing test.

Under the conditions of these experiments, winter wheat plants showed less injury in dry than in wet soil.

The data show no significant differences in the estimated survival between slow and rapid thawing of wheat plants.

In studying the inheritance of cold resistance in winter wheat, over 40,600 F_1 , F_2 , F_4 , and parental plants were subjected to artificial freezing tests, and the estimated survival of each plant determined.

The Poole variety had an estimated percentage survival of 24.1 and 23.3, while Minhardi had an estimated survival of 53 and 51.5 for 1932-33 and 1933-34, respectively. The F_1 plants showed a survival of 36.5 percent.

The estimated survival of the F_2 plants varied from 7.5 to 52.5 percent with an average of 34.6; that is, they were about intermediate to the parents in cold resistance. Families that were less winter-hardy than the nonhardy parent were recombined, which strongly suggests transgressive segregation in the direction of nonwinter hardiness.

The results indicate that cold resistance is inherited in the same manner as other quantitative characters; however, the number of genetic factors involved could not be determined from the data.

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A PHOTOELECTRIC DEVICE FOR THE RAPID MEASUREMENT OF LEAF AREA¹

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Various methods involving the use of light-sensitive cells as reported by Gerdel and Saulter,² Bergman,³ and others for the determination of leaf area have been described. Most of these methods are based on the use of systems of lenses to secure uniform distribution of the light flux over the aperture upon which the leaves are placed.

The use of lens systems to distribute the light from a single source has two inherent limitations aside from the expense. The amount of energy received by the cell or cells is largely limited by the intrinsic brilliancy of the light source, and therefore necessitates the use of sensitive nonportable galvanometers. The size of the lenses employed also sets a definite limit on the size of aperture which can be uniformly irradiated.

The apparatus to be described in this paper was designed to eliminate the difficulties of optical systems employing large lenses by placing the leaves on a ground-glass diffusing screen and irradiating the diaphragm upon which the screen is set with a highly diffused, uniformly distributed source of radiation.

This method is based on the following theoretical considerations: A group of thin opaque objects such as leaves of a total area A is placed upon and in the same plane as a perfect diffusing screen diaphragmed to a known circular area A_o . The aperture of the diaphragm is irradiated from above by a source of such a flux distribution that the intensity of the flux leaving the ground-glass diffusing screen and reaching the sensitive cells is of the same value at all points of the aperture. When this condition exists, the decrease in response of a linear light-sensitive cell, such as a vacuum photoelectric cell, will be proportional to the opaque areas on the aperture of the ground-glass diffusing screen. The following relationship will then obtain:

$$A = LK(I_o - I)$$

$$A_o = KI_o \text{ when } I = 0$$

Where

A_o = Area of diaphragm of diffusing screen

A = Area of leaves

I_o = Photoelectric current with full aperture of the diaphragm

I = Photoelectric current with leaves in place

K = Constant relating photoelectric cell current and area

¹ Received for publication Jan. 18, 1935; issued June, 1935

² GERDEL, R. W., and SAULTER, R. M., MEASUREMENT OF LEAF AREA USING THE PHOTOELECTRIC CELL. *Jour. Amer. Soc. Agron.* 20:635-643. 1928.

³ BERGMAN, H. F., MEASUREMENT OF LEAF AREAS BY MEANS OF THE PHOTOELECTRIC CELL. Report presented at tenth annual meeting Amer. Soc. Plant Phys., Boston, Dec. 30, 1933.

Figure 1 presents in diagrammatic form the details of this instrument, and figure 2 is from a photograph of the apparatus set up for operation.

The illuminator consists of a set of lamp bulbs mounted on an open board above a plate of Florentine or maze glass. The square galvanized-iron housing has a flange at the bottom upon which the glass rests, with a second flange near the top so placed that the lamp bulbs are from 3 to 4 inches above the diffusing glass when the lamp mounting is resting upon it. The lamp mounting consists of 12 receptacles arranged on a board as shown, with a square opening in the center and the corners removed for insuring proper ventilation. The 20 lamp bulbs are mounted in the 12 receptacles by the selection

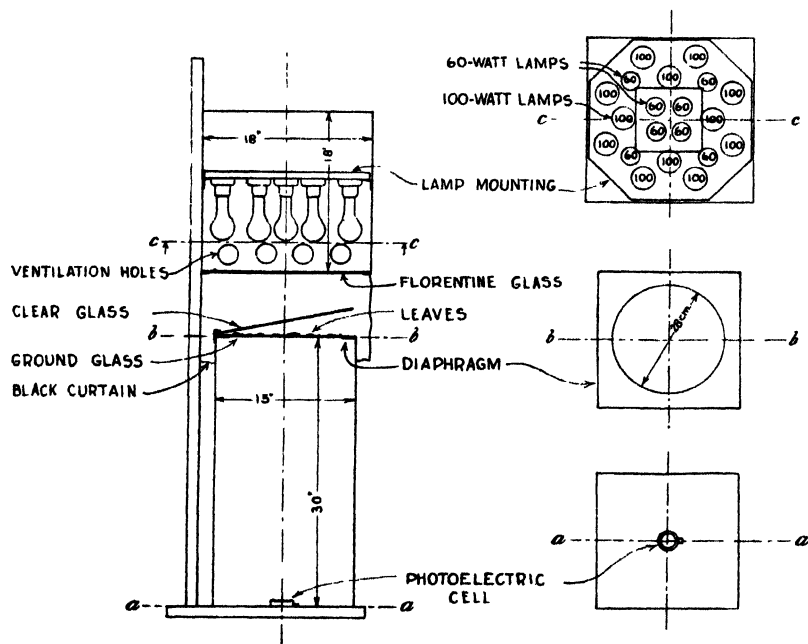


FIGURE 1—Diagrammatic view of leaf-area apparatus in which a photoelectric cell is used.

of suitable Y and offset double sockets. The use of double sockets is not as satisfactory as small receptacles mounted rigidly on an open metal framework. However, they do lend flexibility to an experimental set-up.

The arrangement is not very critical, the only requirement being that the lamps should be evenly distributed over the area, with the higher wattage lamps around the outside. In this manner, the flux is roughly controlled to give a final distribution which is uniform over the surface of the aperture. The final adjustment is made by raising and lowering the illuminator, which is mounted on a wooden frame above the receiving unit. Ventilation is taken care of by a series of 2-inch holes in the lower part of the housing. This is an important factor and must be given careful consideration, for the apparatus consumes nearly 1,700 watts. The sockets should not

have wax plugs around the screws since the heat melts the wax and it runs down onto the bulbs. The inside of the housing may be painted with a heat-resisting aluminum paint which reflects the radiation and prevents the housing from becoming excessively hot.

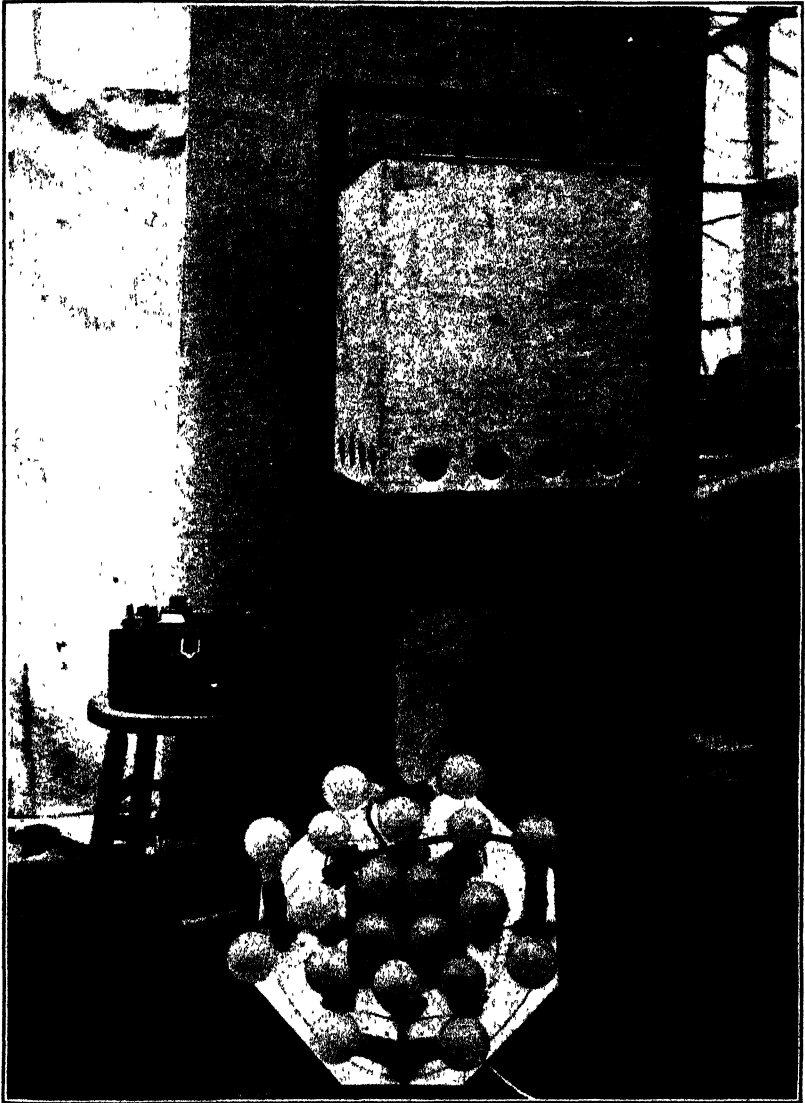


FIGURE 2.—Photograph of leaf-area apparatus set up for operation, with lamp mounting removed from illuminator to show arrangement of lamp bulbs. Potentiometer of portable type on stool.

With one of the earlier instruments, a 1,000-watt lamp in various reflector types was used, but apparently it was not possible to secure the proper distribution of flux with this lamp. A high-wattage lamp would be much more efficient than the lower wattages and much

simpler to use if the proper reflector design could be worked out. Such a reflector would need to give a flux distribution that would be lower in the center than around the periphery of the illuminated area in the plane of the glass-diffusing screen.

The lower receiving unit is smaller in section than the illuminator. The aperture as shown in section *b* of figure 1 is 12 inches in diameter. The diaphragm is covered by a pane of ground glass and a pane of clear glass. The leaves are placed between the two panes and in contact with the ground surface. A flange soldered on the rear of the diaphragm facilitates raising and lowering the clear glass pane for insertion of the leaves.

The light-sensitive unit is mounted in the base. One or more cells may be employed. However, the use of three or more cells increases the over-all accuracy of the instrument, and also makes it possible to install a less sensitive galvanometer. The use of many cells allows a certain degree of adjustment for nonuniform flux distribution, by adjusting the distance of the cells from the aperture and the distance of the cells from one another. It also makes it possible to mount them quite close to the aperture and thus greatly reduce the height of the apparatus.

The determination of flux distribution is made by passing an opaque diaphragm with a small opening over the aperture. It has been found that if the reading on a galvanometer in the cell circuit does not vary more than 10 percent as the opening is moved over the aperture, the resultant accuracy of the entire apparatus will be from 1 to 2 percent of the actual area for areas of from 100 to 500 cm² when a single Weston photonic cell and a small portable galvanometer and potentiometer are used. The control of flux distribution is largely made by raising and lowering the illuminator.

The apparatus is calibrated by taking readings with a series of square or circular pieces of opaque material such as thin metal or black bakelite. These pieces are of known areas. With these data and the value of A_0 and I_0 , a calibration curve can be drawn relating area and photoelectric current.

In practice, the instrument should be calibrated every time the value of A_0 has changed appreciably. The calibration requires only a short time and serves to check the changes in line voltage. Under conditions of seriously fluctuating line voltage, it may be advisable to recalibrate with a few known areas with each area measurement. If the line voltage is constant and the apparatus has had 20 to 30 minutes to come to equilibrium, the A_0 reading will remain constant for long periods. The use of a voltmeter in the line supplying power to the lamps is not of much value, since a change in voltage sufficient to vary the voltmeter reading very slightly will produce a large change in visible radiation emitted by the lamps. The constancy of the electromotive force generated by the light-sensitive cell is the best indication of steadiness of line voltage.

If the response of the cell is not strictly proportional to the intensity of the light falling upon it, the foregoing relationships do not hold and the calibration curve will not be a straight line having a negative slope, but will be somewhat curved. A pair of typical calibration curves exhibiting the linear and nonlinear conditions is shown in figure 3.

These curves were taken with a potentiometer across a photogalvanic type of cell (Weston photronic). In the first case, the cell is operating with a resistance of 50 ohms shunted across it, thus making the response essentially linear. In the second case, no current is flowing through the cell, and the result is the curved or nonlinear voltage response.

The voltage response of the photogalvanic type of cell is high and the internal resistance is relatively low as compared to the photoelectric cell. This makes it well adapted to potentiometric measurements. However, these cells have two unfortunate characteristics. They have a very nonlinear voltage response, the electromotive force falling off from a straight line with increasing light intensities and the curve approaching an asymptote at high values. If a low resistance of less than 50 ohms be placed across the cell, the response becomes essentially

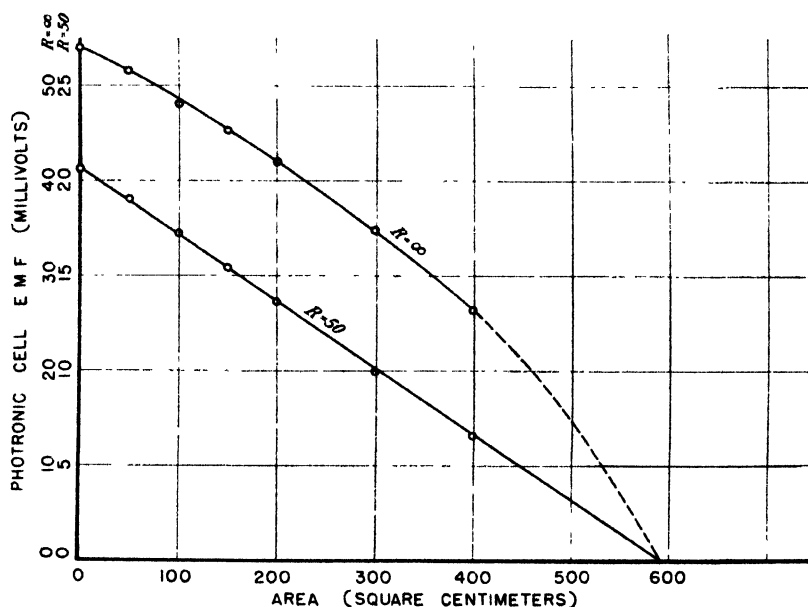


FIGURE 3. —Calibration curves of leaf-area apparatus, showing linear and curved type of responses. R is value of resistor across photronic cell

linear for low light intensities due to the change in internal resistance with light intensity. However, the internal resistance reaches equilibrium rather slowly, and in some cases the last few percentages of change require several minutes to reach their final value. This makes the cell entirely too sluggish for precise work when operating under linear conditions. When a low resistance is used across the cell, the external electromotive force is very greatly reduced, in some cases as much as 90 percent. This adds further to the difficulty of reading. Therefore, it is generally more convenient to use a potentiometer, the reading of which is not influenced by the resistance of the cell.

The output of the photogalvanic type of cell may also be read directly with a sensitive microammeter of fairly low resistance, as is done in the case of some of the direct reading illuminometers which have recently appeared upon the market. With one of these illuminome-

ters or a combination of several photogalvanic cells and a suitable microammeter, the apparatus may be very conveniently calibrated in microamperes or foot-candles.

For the precise determination of area, the use of one or more vacuum photoelectric cells is to be recommended. Under these conditions, the radiation from the Mazda lamps should be more highly and uniformly diffused by the use of two or more layers of diffusing glass spaced an inch or two apart. The modern, well-designed vacuum photoelectric cell, when properly used, is stable and linear in its characteristics. Its main disadvantages lie in the necessity for use of highly sensitive galvanometers or vacuum tube amplifiers.

The leaves of many plants are not, even for practical considerations, entirely opaque and some light passes through them. Thus the areas as read from the calibration chart are incorrect, being lower than the actual value. Therefore, a correction factor must be applied. If the leaves being measured have an average total transmission of T for the light source and cell used, and A' is the observed area, the true area A can be shown to have the following value:

$$A = \frac{A'}{1-T}$$

Thus, the true area is the observed area divided by the absorption of the leaf, the latter often being considerably less than unity.

The light transmitted by most leaves as measured by a photogalvanic type of cell with Mazda lamps for a source varies between 0.05 and 0.15 of the incident light. The transmission may be very conveniently determined by removing the cell from its mounting and placing it in a box of opaque material having a small hole in the lid of such a size that one of the leaves to be studied will readily cover the entire opening. This box is placed on the diaphragm beneath the lamp sources and two readings are taken, one with the leaf in place and one without the leaf. This should be repeated several times and the average of the ratio of each pair of recordings determined. The ratio is the transmission T . For these values, it is essential that the cell be operating under linear conditions.

In order to give an idea of the range of magnitude, the following transmission values of a few typical leaves of greenhouse crops, obtained with a Weston photronic cell, are presented:

Plant:	Transmission T	Plant—Continued.	Transmission T
Aster.....	0.07	Rose.....	0.06
Calendula.....	.08	Stock.....	.06
Leaf lettuce.....	.07	Sunflower.....	.10
Nasturtium.....	.10	Sweetpea.....	.10
Pansy.....	.06	Tomato.....	.12

It is, of course, to be expected that these values are influenced to a large degree by growing conditions. Thus, for each group of determinations, a new value needs to be obtained. Since the correction factor seldom alters the reading more than 15 percent, it is not necessary to determine the transmission of more than a few leaves with a high degree of accuracy.

Leaves are generally quite irregular areas with a large ratio of periphery to area. Therefore, it was thought that an edge effect might be present which would make the apparent area of a large number of irregular small surfaces different from that of one large surface of equal area. To test this possibility, a square piece of opaque black paper of 400 cm² area was placed on the diaphragm and a reading taken. The paper was then cut into a large number of small irregular pieces to simulate leaves and a reading again taken. The test was repeated several times and in no case did the second reading differ from the first by more than a few percent. This indicates that no appreciable edge effect exists.

Although the apparatus which has just been discussed is suitable only for detached leaves, it would be readily possible to adapt the same principles to the design of a small portable unit for measuring leaves on the plant. Such a device could be made with a pair of 45-degree mirrors so placed that the Mazda lamps and the photoelectric cells would not interfere with the remaining portion of the plant.

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A PHYSIOLOGICAL STUDY OF DEVELOPMENT AND RIPENING IN THE STRAWBERRY¹

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INTRODUCTION

The work reported in this paper constitutes a comparative study of the physiology of development and ripening in a group of varieties of strawberries (*Fragaria* spp.) so selected as to represent the most popular and widely grown commercial varieties of the Atlantic seaboard States. It was begun in 1925 and was continued for 4 years in conjunction with a more extensive study of a larger number of strawberry varieties and strains which was being carried on to determine their suitability for the fresh market and for canning, preserving, freezing, and other methods of preservation. So far as the writers have been able to ascertain, no detailed study of the physiology of development of the strawberry has hitherto been made. Such reports of chemical analyses of the fruit as were found in the literature were concerned with the composition of the mature fruit, and these were few, by far the larger number of the named varieties in general cultivation apparently never having been subjected to analysis for any purpose.

The present work attempts to extend existing knowledge of the physiology of the fruit by outlining the nature of the chemical and physical changes that take place in it during the course of development and ripening. A considerable number of varieties have been employed for purposes of comparison.

REVIEW OF LITERATURE

Trzeciok (21)³ in 1892 reviewed such analytical work on the strawberry as had appeared in the European literature up to that time, and reported the results of his own determinations of total solids, titratable acidity, reducing and total sugars, and ash upon material obtained from wild plants growing in the vicinity of Erlangen, Bavaria. Detailed analyses of the ash of the fruit were made. The sugar and the acid content of the freshly expressed juice were determined, the juice was subjected to fermentation by yeast, and the analysis of the liquid was repeated at intervals during the course of the fermentation. Most of the analyses cited by Trzeciok deal with the wild European strawberry, *Fragaria vesca* L., as do those assembled by König (16, pp. 776-777), and in work dealing with cultivated varieties the varietal names are not given.

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² The writers desire to express their gratitude to George M. Darrow, senior pomologist, Division of Fruit and Vegetable Crops and Diseases, for permission to use the material employed in this study and for many courtesies in the course of the work.

³ Reference is made by number (italic) to Literature Cited, p. 695.

A number of earlier American workers have reported the results of analyses made primarily for the purpose of comparing the nutritive value of the strawberry with that of other fruits. Stone (20) made analyses of 20 varieties of ripe strawberries grown at the Tennessee Agricultural Experiment Station in 1889, the determinations including total solids by drying at 105° C., reducing and total sugars, acidity as malic acid, crude fiber, crude protein, ether extract, and nonnitrogenous extract. Shaw (18) made analyses of 9 varieties grown at the Oregon Agricultural Experiment Station in 1898 and 1899, and compared the averaged results with those reported by Stone and with those for European varieties assembled by König. Shaw reached the conclusion that the European varieties are somewhat higher in sugar and acid and in insoluble and total solids than American berries, but are lower in protein. The comparison would appear to be essentially a comparison of the European *Fragaria vesca* with the American *F. virginiana* Duchesne, with or without hybridization with other species. Weber (23) made determinations of acid and sugar content upon 2 varieties, one an unnamed seedling, at the Ohio Agricultural Experiment Station in 1887. Of all the varieties named by these workers, not more than two or three are now being propagated by nurserymen and none is important commercially.

The only recent work approximating a comparative study of the chemistry of a group of varieties is that of Vercier (22), which was concerned primarily with the determination of the effect of domestication and hybridization in altering size and composition of the fruit. Vercier grew 15 cultivated varieties and strains of strawberries under identical conditions together with the wild *Fragaria vesca* and made determinations of weight per berry; percentage of dry matter, sugar, and acid in the fruit; and yield of juice. As compared with the wild species, the cultivated strains were 600 to 1,800 percent higher in average weight per berry, but were markedly lower in total solids and sugars, and in most cases somewhat lower in titratable acidity. Vercier concluded that the trend of selection and breeding had been toward increase in size of fruits at the expense of the solids content and food value of the fruit. Gimingham (12) grew 2 varieties of strawberries, Epicure and Royal Sovereign, on 5 types of soil, continuing the work over 3 years and determining percentages of sugar and acid in the fruit. He concluded that each variety has a definite standard of composition that is affected in only a very limited degree by the character of the soil on which the berry is grown. Royal Sovereign was consistently higher in sugar content and lower in titratable acidity than Epicure on all soils and in all seasons. In the degree of influence exerted upon sugar content, seasonal conditions were predominant over all other factors.

Studies of the effects of cultural treatments or fertilizer applications upon growth and productiveness of the strawberry plant are rather numerous but rarely include analytical data upon the fruit. The work of Shoemaker and Grove (19) presents an exception. These authors determined the effects of rather heavy spring applications of a nitrogenous fertilizer on the fruit of only one variety, namely, Howard 17 (Premier). The fruit from the heavily fertilized plots was slightly less firm as determined by resistance to pressure tests, was consistently higher in total nitrogen (including nitrates, which were

not separately determined), higher in catalase activity, lower in actual acidity and in reducing sugar, and generally lower in total sugars than that from the check plots. There were no consistent differences in moisture content or in ability of the fruit to withstand handling and shipment. Kimbrough (15), working at the Alabama Agricultural Experiment Station, found that weather conditions, especially rainfall, had a decidedly greater effect upon the composition of strawberries of the Missionary ⁴ variety than did fertilizer treatments. He determined the moisture and sugar content of berries from the various fertilizer plots at intervals of a few days throughout the bearing season. The amount of rainfall for 3 to 6 days prior to picking determined the composition, as berries from all plots had high moisture content and low sugar content after rainfall, and the reverse condition after a period of no precipitation. In a practically rainless fruiting season, fruit from all plots artificially watered was uniformly softer in texture than that from unwatered plots. Cochran and Webster (4) made analyses of berries of the variety Aroma ⁵ at the Oklahoma Agricultural Experiment Station from 12 plots that had received various fertilizer treatments, but were unable to find consistent correlations between composition and firmness or between either of these factors and fertilizer treatments. Loree (17) made detailed analyses of nitrogen, carbohydrate, and mineral content of roots and tops of Dunlap strawberry plants after the application of various fertilizer combinations, but confined his analytical work on the fruit to determination of moisture content. A number of workers have reported results of determinations of the nutritive value of the strawberry or of examination of the fruit for the presence or absence of some particular constituent, but review of work of this character is not pertinent to the present purpose.

MATERIAL AND METHODS

The material employed in the study was grown at the United States Plant Field Station at Glenn Dale, Md., and formed a part of the variety collection assembled in connection with the strawberry-breeding work conducted by George M. Darrow. The Glenn Dale station is located 15 miles northeast of Washington, D. C. The soil formation is classified as Collington sandy loam, the particular area occupied by the strawberry plantings being a decidedly sandy loam. Rearrangement of the plantings at the station in 1927 necessitated the transfer of the variety collection to another area, but the conditions in the new location were not materially different from those in the old. The samples taken in 1928 were obtained from this planting.

Twelve varieties, namely, Missionary, Howard 17 (Premier), Klondike, Parsons (Gibson), New York, Dunlap, Sample, Aroma, Gandy, Chesapeake, Progressive, and Portia, were employed in the study. The first 9 are the most widely grown commercial varieties, and together made up more than 80 percent of the commercial acreage of the United States at the time the work was begun (8, p. 308). Chesapeake is of considerable commercial importance in Maryland and adjacent territory, Progressive is one of the most widely grown everbearing strawberries, and Portia is a canning variety of relatively

⁴ Name of variety was supplied by Dr. Kimbrough in a personal communication.

⁵ Name of variety supplied by Professor Cochran in a personal communication.

restricted cultivation that has shown promise of value. Collectively, the group was believed to be as truly representative of the commercially important strawberries of the country as it is possible for material grown at any one location to be.

The general plan followed in obtaining material for analysis was to make collections of fruit at intervals of 5 to 7 days during the period of development and ripening. This method of sampling secured from each variety in every year two fairly complete series of fruits, one developed from the earlier, the other from the later blooms. The first collection was generally made as soon after blooms had set as sufficient material could be obtained, and usually consisted of berries that were just dropping the petals but which differed considerably in size. These for the most part had developed from the first flowers to open on the plants. Upon arrival at the laboratory, the berries were graded into 2 or 3 lots on the basis of size, if the quantity of available material and the range in size of fruit permitted such separation. It was originally intended to begin the sampling of any variety as soon as the plants had set sufficient fruit to yield representative samples, but this was found to be impracticable. The list of varieties includes early, midseason, and late-blooming, but the actual sequence of flowering varied greatly from year to year. As the distance from the laboratory precluded the making of collections with sufficient frequency to secure individual varieties at any desired stage, the method adopted was that of beginning sampling as soon as a majority of the varieties had set sufficient fruit. Consequently the initial samples varied somewhat in size and stage of development from year to year.

The second sampling was usually made approximately a week after the first. At that time an attempt was made to collect material representative of all the fruit on the plants, large and small. This material was subsequently divided into two or three lots on the basis of size, and a similar method was followed at subsequent samplings. In consequence the smallest fruits collected at the second or third sampling more or less closely approximated in size and stage of development the largest fruits taken at the first sampling. Similar duplications occurred throughout, so that the season's samples were made up of two or three series which succeeded one another in development on the plants and collectively constituted a series of cross sections at various stages of development of the crop borne by the plants in that year. As the fruit became full-grown and ripening set in, the material was divided on the basis of color, as green, whitened, reddened, and ripe, without reference to size.

This method of sampling affords no information as to the exact age of the fruits making up any particular sample. At the time the plants were flowering it was not feasible to tag flowers at opening so that the exact age of fruits could be known; consequently it was necessary to rely on the size and color of the fruit as a means of grading it roughly into lots of approximately equal age. For determining stages of maturity only slightly differing one from another it was necessary to rely upon the puncture tests.

The firmness, or resistance of the fruit to puncture, was determined as soon as the fruit reached the laboratory. The pressure tester described by Culpepper and Magoon (6) was used, with a needle 0.932

inch in diameter and a scale graduated in 5-g intervals. Twenty to thirty berries were used in a test, each being tested at 2 to 4 points, midway from stem to tip, at the tip, and near the attachment to the stem. The values given in table 1 under the heading "Puncture tests" are averages of all the readings obtained from the individual tests made upon the sample.

The instrument employed for measuring the firmness of the fruit was not ideal in every respect. It does not give a measure of the resistance of the fruit to deformation or flattening as does the pressure or "squeeze" tester developed by Haller, Harding, and Rose (13) subsequent to the completion of this work. It measures only the resistance of the tissues to penetration by a small needle. As shown later, this type of tester gives an index of maturity which is quite dependable, but it does not bring out varietal differences in firmness or resistance to crushing in shipping or handling.

Determinations of hydron concentration were made, in 1927 and 1928 only, on a portion of the fresh sample. The berries were finely ground in a mortar and the juice was expressed by hand through triple layers of muslin. Duplicate or triplicate determinations, agreeing to within 0.05 pH, were made by means of the Bailey hydrogen electrode. A quinhydrone electrode was employed as a comparison electrode. In some cases scarcity of material necessitated the use of a micro-electrode (2). In these instances, a number of agreeing determinations were always secured.

Samples for chemical analysis were made in duplicate and were preserved by slicing into a tared beaker a sufficient number of berries to give the desired weight (50 or 100 g), transferring them to a storage container, adding sufficient 95 percent alcohol to make the final concentration 80 to 85 percent, heating to boiling, sealing, and storing in the laboratory until the analyses were begun, usually after an interval of 6 to 8 months. At that time extraction was completed with fresh 95-percent alcohol in a Soxhlet extractor, the extract made up to volume, and aliquots taken for the several determinations, which were made according to the official methods of the Association of Official Agricultural Chemists (1).

ANALYTICAL DATA

The analytical data are presented in table 1. In the examination of these data the purpose will be (1) to trace in general outline the character of the changes in composition occurring in the fruit in the course of its development, regardless of variety or year; and (2) to determine the extent to which a variety has definable chemical individuality which persists from season to season despite variations in seasonal conditions and other varying factors and which consequently characterizes the variety as a chemical and physical entity. The data should indicate the extent to which the course of these changes may be modified by the environmental conditions prevailing in the plots during the development of the fruit, but since detailed records of these conditions were not kept, a discussion of this phase of the subject is not attempted.

TABLE 1.—Chemical composition and resistance to puncture of strawberry varieties during development and ripening

AROMA

Date	Diameter	Description	Sugar		Titratable acidity	Astringency		Solids		Puncture test	pH			
			Reducing	Cane		Total	Total	Tannin	Nontan- nin			Soluble	Insolu- ble	Total
1925														
May 13	Mm		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Grams				
Do.	8-9		1.70	0.32	2.02	0.659	2.179	1.504	0.675	5.30	8.30	13.60	378	
Do.	8-12		2.29	.24	2.53	.906	1.248	.735	.513	4.96	7.34	12.30	354	
May 19	9-15		1.98	.02	2.00	.688	.695	.201	.994	5.66	7.37	13.03	330	
Do.	15-21	Large red	2.44	.18	2.62	.771	.575	.268	.207	5.29	5.89	11.18	293	
May 26		Green, reddened	2.23	.70	2.93	.640	.436	.248	.198	5.06	4.54	9.60	213	
Do.		Medium red	2.60	.62	3.22	.569	.332	.185	.177	5.20	3.67	8.87	110	
Do.		Red ripe	3.29	.25	3.54	.480	.324	.116	.218	5.64	3.50	9.14	80	
June 5		Very soft	4.68	.30	4.98	.439	.347	.183	.164	6.03	3.38	9.41	12	
1926														
May 27	8-11	Small	2.00	.54	2.54	.735	1.526	1.144	.382	5.96	9.40	15.36	356	
Do.	11-15	Medium	2.32	.65	2.97	.796	1.348	.985	.453	5.82	7.54	13.42	302	
Do.	12-18	Large	2.90	.18	3.08	.909	.988	.761	.227	5.88	7.27	13.09	215	
June 3	18-20	Green	3.38	.51	3.89	.724	.428	.284	.145	5.77	6.73	12.50	38	
Do.	24-32	Medium	3.73	.65	4.38	.706	.392	.235	.157	6.14	3.55	9.69	32	
Do.	24-35	Ripe	4.68	.46	5.14	.679	.563	.469	.094	7.18	3.43	10.61	16	
June 11	24-35	Full ripe	4.51	.66	5.17	.559	.300	.132	.168	6.68	3.02	9.70	15	
1927														
June 3	18-27	5 percent red	2.59	.36	2.95	.990	.438	.260	.178	5.28	5.13	10.41	101	
Do.	18-27	75 percent red	2.56	.84	3.40	.990	.400	.250	.150	5.52	4.09	9.61	61	
Do.	18-27	Firm ripe	3.52	.84	4.36	.811	.250	.114	.166	6.88	3.11	9.99	39	

CHESAPEAKE

1925													
May 13.....	6-12		1.80	1.15	2.95	0.972	3.662	1.796	1.796	1.866	6.30	10.34	16.64
Do.....	9-15		1.97	.45	2.42	.974	2.048	.824	.824	1.224	5.21	8.11	13.32
May 19.....	6-12		2.01	.51	2.52	1.376	2.726	1.205	1.205	1.521	5.92	8.36	14.28
Do.....	9-15		2.76	.42	3.18	.903	1.325	1.061	1.061	.244	6.02	6.81	12.83
May 26.....		Green, reddened	3.61	.63	3.94	.685	.394	.218	.218	.176	6.08	4.43	10.51
Do.....		Red ripe	3.82	.89	4.71	.671	.297	.161	.161	.136	6.52	3.61	10.13
June 5.....		Ripe	5.38	.19	5.57	.573	.363	.188	.188	.175	6.46	3.37	9.83

TABLE 1.—*Chemical composition and resistance to puncture of strawberry varieties during development and ripening—Continued*

DUNLAP—Continued

Date	Diameter	Description	Sugar			Titratable acidity	Astringency			Solids			Puncture test	pH
			Reducing	Cane	Total		Total	Tannin	Non-tannin	Soluble	Insoluble	Total		
			Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Grams	
1927	M/m	Green	1.25	.23	1.48	.910	1.070	.652	.418	4.48	8.79	13.27	941	3.68
	May 12	11-14	1.25	.39	1.64	.883	.740	.476	.264	4.08	8.15	12.23	941	3.68
	May 20	11-14	1.13	.35	1.52	.883	.510	.300	.210	4.04	7.28	11.32	256	3.57
	Do.	12-15	1.41	.12	1.53	.942	.482	.276	.206	4.20	6.83	11.03	290	3.57
	Do.	15-20	1.54	.37	1.92	1.062	.378	.230	.148	4.15	4.92	9.07	290	3.35
	May 28	18-24	1.92	.37	2.29	1.062	.378	.230	.148	4.15	4.92	9.07	290	3.35
1928	Do.	25 percent pale red	2.28	.30	2.58	1.030	.274	.210	.080	5.72	3.64	9.36	51	3.43
	Do.	Fairly deep red	3.22	.46	3.68	.942	.290	.242	.148	5.72	3.64	9.36	51	3.43
	18-27	Dead ripe	3.32	1.10	4.42	.740	.254	.128	.126	6.20	3.08	9.28	26	3.34
	June 3													
GANDY														
1925	5-8		2.09	.20	2.29	1.280	4.109	2.507	1.602	7.13	11.21	18.34	439	---
	6-12		2.10	.52	2.62	1.036	2.512	1.143	1.360	5.12	7.88	13.00	423	---
	9-15		1.58	.66	2.24	1.247	2.512	1.143	1.360	5.37	8.54	13.91	410	---
	12-21		2.06	.51	2.57	1.363	1.595	.890	1.106	5.27	8.37	13.64	373	---
	May 26	Green	2.65	.34	2.99	.760	.569	.210	.359	5.36	5.91	11.27	297	---
	Do.	Red ripe	3.45	.45	3.90	.845	.418	.242	.176	6.60	4.49	11.18	158	---
1926	June 1	Medium firm	4.85	.58	5.43	.744	.382	.188	.194	7.07	3.74	10.81	25	---
	May 27	Small	2.59	.20	2.79	.845	1.752	1.476	.276	6.37	10.11	16.48	330	---
	Do.	Large	2.60	.38	2.98	.917	1.430			6.67	8.97	15.65	317	---
	June 3	White	3.48	.22	3.70	1.271	.915	.711	.204	7.00	6.41	13.41	131	---
1927	18-30	Full ripe	6.39	.84	7.23	.840	.433	.225	.206	9.30	3.58	12.88	15	---
	May 28	Small	1.51	.59	2.10	1.257	1.140	.740	.400	5.76	10.45	16.21	335	---
	Do.	Medium	2.00	.47	2.47	1.268	.604	.604	.238	5.80	8.56	14.36	285	---
	21-27	Large	2.26	.64	2.90	1.327	.666	.464	.182	5.80	6.77	12.57	218	---
	Do.	White	3.13	1.07	4.20	1.620	.480	.334	.146	6.92	5.29	12.21	107	---
	24-36	75 percent red	4.06	1.27	5.33	1.525	.362	.222	.140	8.12	3.98	12.10	51	---
1928	27-36	Firm ripe	4.56	1.79	6.35	1.331	.338	.218	.120	8.68	3.53	12.21	26	---

HOWARD 17 (PREMIER)

1925	6-9	9-12	12-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	70-75	75-80	80-85	85-90	90-95	95-100	100+
Apr 28	1.97	0.30	2.27	5.82	1.226	3.396	2.741	0.655	5.17	8.59	13.76	317									
Do.	1.83	0.27	2.58	5.82	1.219	3.04	1.559	0.649	4.53	7.64	12.03	321									
May 13	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 19	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 26	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 28	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	2.02	0.57	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
1926	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 21	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 27	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
June 3	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
June 8	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.76	0.82	2.58	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
1927	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 12	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 20	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 28	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
June 3	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.90	0.38	2.18	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
1928	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 17	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
May 29	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
June 11	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									
Do.	1.50	0.57	2.16	5.82	1.219	1.702	1.559	0.649	4.53	7.64	12.03	321									

MISSIONARY

1925	Apr. 28	3-9	0.67	1.07	1.74	1.342	3.922	2.628	1.294	5.37	10.31	15.68	372	
	Do.	6-12	2.07	.35	2.42	1.254	4.050	2.788	1.262	5.36	9.42	15.28	343	
	May 13	9-12	1.58	.87	2.45	1.259	2.453	1.801	.652	5.56	9.40	14.56	336	
	Do.	9-18	2.27	.15	2.42	.847	1.937	.585	.352	5.36	6.68	12.04	320	
	Do.	15-21	2.20	.71	3.91	1.000	.803	.574	.229	6.45	4.20	10.65	287	
	May 19	6-15	2.00	.42	2.92	1.040	1.221	.634	.567	5.52	5.09	10.61	241	
	Do.	18-30	3.41	.54	3.95	.935	1.332	.132	.213	6.74	3.70	10.44	116	
	Do.	18-30	4.27	.82	5.09	.760	.269	.065	.204	7.54	4.27	11.81	34	
	May 26	Very ripe	5.01	.72	5.73	.505	.404	.179	.225	7.13	3.46	10.59	20	
	1926	May 21	6-12	1.81	.82	2.63	.940	2.002	1.071	.931	6.05	8.93	14.98	339
		Do.	11-15											337
Do.		Large	2.36	1.38	3.74	.982	1.192	.862	.330	5.73	6.59	12.32	230	
May 27		14-15	2.85	.69	3.54	.674	.748	.462	.296	5.84	5.62	11.46	196	
Do.		21-27	3.43	.81	4.24	.832	.568	.422	.166	6.88	4.16	11.04	66	
Do.		24-33	4.30	.36	4.66	.852	.555	.421	.134	6.75	3.46	10.21	35	
June 3		17-27	2.97	.53	3.50	.953	.523	.365	.158	5.69	3.93	9.62	39	
Do.		18-30	3.57	.34	3.91	.954	.402	.237	.165	6.10	3.42	9.52	24	
Do.		18-30	4.56	.41	4.97	.685	.379	.217	.162	6.94	3.05	9.99	12	
1927		May 28	3-9	1.54	.16	1.70	1.123	1.194	.729	.465	4.98	10.55	15.53	338
		Do.	9-18	1.41	.41	1.82	1.250	.780	.504	.276	4.96	7.75	12.71	288
	Do.	12-21	1.88	.54	2.42	1.322	.570	.360	.210	5.36	5.91	11.27	161	
	June 3	12-24	2.57	.19	2.76	1.297	.478	.343	.133	5.24	4.82	10.06	128	
	Do.	Ripe	2.95	.45	3.40	1.285	.420	.240	.130	6.16	4.14	10.30	69	
	Do.	do.	5.22	.62	5.84	1.048	.340	.208	.132	8.24	3.59	11.83	27	

NEW YORK

1925												
May 5.	8-11	1.66	0.51	2.17	1.610	3.667	1.971	1.696	5.31	8.97	14.28	446
Do.	11-14	3.31	.84	4.15	1.033	2.128	1.308	.820	5.37	9.06	14.43	403
May 13.	6-15	2.38	.21	2.59	.601	1.239	.793	.446	5.63	8.13	13.76	382
Do.	12-18	2.25	.60	2.85	.817	.744	.520	.224	5.20	6.49	11.69	313
May 19.	12-24	2.77	.20	2.97	.795	.345	.096	.279	5.82	5.45	11.27	212
Do.		3.64	.56	4.20	.933	.304	.294	.130	6.89	4.10	10.99	97
May 26.		4.25	1.01	5.26	.650	.292	.145	.147	7.62	3.57	11.13	45
Do.		4.72	1.34	6.06	.611	.274	.125	.149	8.02	3.23	11.85	25
June 4.		6.70	1.03	7.79	.552	.276	.116	.160	9.02	3.43	12.45	10
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† Tinge of red on a few.

TABLE 1.—Chemical composition and resistance to puncture of strawberry varieties during development and ripening—Continued

NEW YORK—Continued

Date	Diameter	Description	Sugar			Titratable acidity	Astringency				Solids			Puncture test	pH
			Reducing	Cane	Total		Total	Tannin	Non-tannin	Soluble	Insoluble	Total			
1926	M/m		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Grams	
	8-15	Small	1.70	.60	2.30	.958	2.016	1.545	.471	.572	6.17	12.68	18.85	420	
	19-24	Large	2.03	.76	2.79	.880	1.254	.682	.572	5.62	9.33	14.95	350		
	14-17	Small	2.61	.51	3.12	.872	.765	.534	.231	5.72	7.35	13.07	211		
	18-39	Medium	3.15	.41	3.56	.874	.628	.463	.537	6.25	6.50	12.75	204		
	21-38	Large	4.03	.70	4.73	.849	.387	.209	.178	7.18	4.15	11.33	56		
	42	Green	4.38	.41	5.07	1.027	.310	.174	.136	8.76	3.62	12.38	16		
	24-48	Underripe	5.96	1.34	7.30	1.706	.300	.102	.198	7.54	2.39	9.93	10		
	24-48	Full ripe	4.75	.69	5.44	.658									
	1928														
May 17		5-9	1.75	1.45	3.20	1.040	2.440				6.40	16.52	22.92	400	4.31
Do.		8-12	1.72	1.08	2.80	.928	1.708				6.00	10.68	16.68	380	4.02
May 29		17-23	2.14	.80	2.94	1.000	.482				5.32	6.33	11.65	176	3.39
Do.		20-30	2.82	.76	3.58	1.390	.352				6.08	5.19	11.27	142	3.24
June 11		20-30	4.80	1.26	6.06	.755	.217				8.38	3.30	11.68	16	3.57

PARSONS

1925														
May 5	5-6		1.70	0.57	2.27	0.980	4.975	3.370	0.905	6.17	10.55	16.72	420	
Do.	8-12		2.20	.80	3.00	1.157	3.131	2.224	.907	6.00	8.38	14.38	386	
May 13	8-11		1.67	.45	2.12	.906	2.303	1.741	.946	5.14	8.24	13.38	372	
Do.	9-15		1.73	.44	2.17	1.523	1.807	.683	1.14	4.79	6.67	11.46	285	
May 19	9-15		1.90	.17	2.06	1.012	1.083	.853	.231	5.05	6.49	10.54	265	
Do.	18-24		2.10	.867	2.96	1.012	1.488	.271	.231	5.05	6.49	10.54	265	
May 26	26	Green, reddened	2.68	.62	3.30	.694	.381	.204	.177	5.08	3.62	9.50	202	
Do.	Red ripe		3.02	.53	3.55	.714	.350	.172	.178	5.82	3.18	9.00	61	
June 5	5	Very soft	4.61	.44	5.05	.533	.350	.158	.192	6.36	3.32	9.68	16	
1926														
May 21	5-8	Small	2.35	.74	3.09	.829	2.130	1.232	.878	6.14	9.15	15.29	400	
Do.	8-9	Large	2.62	.25	2.87	.852	1.550	.977	.573	5.63	8.07	13.70	310	
May 27	15-21	Small	4.46	.51	4.97	1.026	1.158	.992	.166	5.78	7.36	13.14	253	
Do.	12-20	Large	3.85	.48	3.33	1.711	.738	.503	.235	5.81	6.17	11.98	194	
June 3	22	Medium	3.68	.71	4.39	1.270	.413	.228	.175	6.44	4.08	10.52	26	
Do.	32	Ripe	4.39	.69	5.08	.738	.428	.332	.096	6.76	3.13	9.89	19	

1927		PORTIA										1925		1926										1927		1928																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
June 3	Green.....	1.64	1.18	2.82	1.216	.621	.374	.247	6.32	4.67	10.99	151	May 5	6-9	2.17	1.08	3.25	1.855	5.406	3.858	1.548	6.29	10.72	17.01	405	May 17	5-11	1.36	1.32	2.68	1.852	780	1.852	6.24	16.08	22.32	334	4.04	May 20	15-21	1.59	.35	1.94	800	882	612	270	8.46	8.46	13.06	262	May 28	15-21	2.04	.64	2.58	983	.095	.296	230	5.44	5.98	11.42	187	June 3	18-24	2.51	.76	3.11	1.010	.400	.330	170	5.44	5.88	11.32	110	June 8	18-24	2.64	.80	3.62	852	.340	.210	130	5.84	3.86	9.70	51	June 11	18-24	2.70	.80	3.52	948	.330	.220	110	5.56	4.17	9.73	51	June 14	18-24	2.70	.80	3.52	700	.296	.144	122	5.72	2.43	8.15	28	June 17	18-24	2.70	.80	3.52	683	.250	.104	146	6.48	3.40	9.88	22	June 20	18-24	2.75	1.15	4.93										June 23	18-24	3.75	1.15	4.93										June 26	18-24	3.75	1.15	4.93										June 29	18-24	3.75	1.15	4.93										June 31	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 24	18-24	3.75	1.15	4.93										June 27	18-24	3.75	1.15	4.93										June 30	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 24	18-24	3.75	1.15	4.93										June 27	18-24	3.75	1.15	4.93										June 30	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 24	18-24	3.75	1.15	4.93										June 27	18-24	3.75	1.15	4.93										June 30	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 24	18-24	3.75	1.15	4.93										June 27	18-24	3.75	1.15	4.93										June 30	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 24	18-24	3.75	1.15	4.93										June 27	18-24	3.75	1.15	4.93										June 30	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 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6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 24	18-24	3.75	1.15	4.93										June 27	18-24	3.75	1.15	4.93										June 30	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 24	18-24	3.75	1.15	4.93										June 27	18-24	3.75	1.15	4.93										June 30	18-24	3.75	1.15	4.93										June 3	18-24	3.75	1.15	4.93										June 6	18-24	3.75	1.15	4.93										June 9	18-24	3.75	1.15	4.93										June 12	18-24	3.75	1.15	4.93										June 15	18-24	3.75	1.15	4.93										June 18	18-24	3.75	1.15	4.93										June 21	18-24	3.75	1.15	4.93										June 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TABLE 1.—Chemical composition and resistance to puncture of strawberry varieties during development and ripening—Continued

PROGRESSIVE

Date	Diameter	Description	Sugar		Titratable acidity	Astringency		Solids		Puncture test	pH
			Reducing	Cane		Total	Tannin	Non-tannin	Soluble		
			Percent	Percent	Percent	Percent	Percent	Percent	Percent	Grams	
1925	May 5.....	5-9	1.60	0.68	0.929	2.700	2.024	0.766	5.58	15.69	---
	Do.....	8-11	1.64	1.17	1.179	2.109	1.639	0.470	8.92	14.42	---
	May 13.....	9-15	1.65	1.16	1.179	2.109	1.639	0.470	8.92	14.42	---
	Do.....	12-18	2.35	2.27	0.926	2.821	3.08	376	4.45	15.66	---
	Do.....	12-21	2.89	2.74	0.940	3.02	1.03	1.99	5.65	15.66	---
	May 19.....	15-21	2.68	2.43	0.812	3.42	1.03	1.99	6.18	10.64	---
	Do.....	18-27	2.79	2.77	0.800	2.50	1.04	1.14	5.74	10.21	---
	Do.....	18-30	2.67	3.1	0.96	3.65	1.40	226	5.88	9.47	---
	May 26.....	Green.	3.58	63	500	384	247	137	4.82	8.20	---
	May 27.....	Very ripe.	3.96	75	522	384	247	137	5.76	8.60	---
1926	May 21.....	6-11	1.70	1.14	783	1.420	1.133	287	5.31	13.88	---
	Do.....	11-17	2.17	1.31	830	864	669	195	5.00	12.00	---
	Do.....	17-23	3.43	3.43	882	600	307	263	5.14	10.83	---
	May 27.....	Green.	2.71	1.00	810	540	380	160	5.19	10.06	---
	Do.....	12-24	3.46	41	805	410	316	094	6.07	9.74	---
	Do.....	Medium.	4.22	41	706	328	247	081	6.80	9.96	---
	June 3.....	Ripe	2.54	13	787	383	186	197	5.30	9.26	---
	Do.....	Greenish red	3.21	80	546	243	175	098	5.16	9.26	---
	May 27.....	Medium	3.79	49	452	312	172	140	5.92	8.41	---
	June 8.....	Ripe	2.97	59	464	266	130	136	5.06	8.90	---
1927	May 20.....	11-15	1.67	1.6	936	839	504	335	4.57	12.17	3.46
	Do.....	14-18	1.90	45	994	562	240	240	4.56	11.11	3.38
	May 28.....	White	2.06	1.06	1,160	350	232	118	5.80	10.20	3.23
	Do.....	15-21	2.81	1.04	1,050	274	164	088	5.84	9.67	3.22
	Do.....	60 percent red	2.80	1.14	911	238	150	088	6.20	9.40	3.19
	June 3.....	Deep red	2.60	1.11	1,218	340	190	150	6.20	10.01	3.19
	Do.....	Red	3.97	1.92	852	324	206	118	7.32	10.46	3.27
	Do.....	18-27	3.97	1.92	852	324	206	118	7.32	10.46	3.27
	May 17.....	1928	1.62	.90	884	1,650	---	---	5.44	14.62	4.24
	June 11.....	Ripe	4.16	2.25	669	213	---	---	7.88	13.83	3.37

GENERAL COURSE OF PHYSIOLOGICAL CHANGES DURING DEVELOPMENT

FIRST SAMPLING

For reasons already stated, the size and age of the berries making up the initial samples varied considerably from variety to variety and from year to year. Considered collectively, the initial samples of 1925 and 1928 were very nearly identical in size and presumably in age; those of 1926 and 1927 were distinctly larger.

In common with a number of fruits of varied types (3), the young strawberry shows very definite and consistent chemical and physical characteristics. When compared with the later stages, the initial samples of all varieties in all years are characterized as a group by the possession of maximum resistance to puncture, maximum total solids, maximum insoluble solids, and maximum total astringency. For reasons discussed later, the absolute values for all these constituents vary considerably from variety to variety and from year to year, but without exception the smallest sample of any variety taken in any given year had a maximum content of each of these constituents which progressively declined through the series of samples subsequently taken. Further, the maximum values for total and insoluble solids found anywhere in the series were present in the initial samples of New York, Portia, and Chesapeake, in 1928, which were taken when most of the berries had some of the petals still adhering and which were judged to be the youngest as well as the smallest berries obtained in the course of the work.

The total solids of the young fruits are characterized by the preponderance therein of insoluble solids, which make up 60 to 70 percent of the total, the percentage being maximum in the youngest berries obtained (Portia and New York, 1928).

The soluble solids are quite variable in composition. The outstanding and constant characteristic is the very large amount of astringent material as indicated by the usual method of titration with potassium permanganate and calculation in terms of the reducing power of chestnut tannin. In all the very young samples, permanganate-reducing substances when calculated as tannins make up 30 percent or more of the soluble solids, or more than 10 percent of the total solids (Aroma, 1925; Chesapeake, 1925, 1928; Dunlap and Gandy, 1925; Missionary, 1925, 1926). The nature of this material is problematical and its statement in terms of the conventional tannin factor serves for convenience in comparison only. That it is actually much smaller in amount than these figures indicate is shown by the fact that for the youngest stages the sum of total sugars, titratable acids, and astringents calculated as tannin exceeds the total sum of soluble solids as determined by drying the alcoholic extract. All that can be said at present is that the young fruit is characterized by the presence of considerable quantities of readily oxidizable substances which are most abundant in the very young fruit and which decrease with age.

The nontannin fraction of the astringent material (portion not precipitable by gelatin) varies rather widely both in absolute amount and in the proportion it forms of the total astringents, but in every instance it is largest in the first sample of a series. Both total and nontannin astringency show very large and abrupt decreases with increase in age

and size of berries. This behavior they share with a variety of fruits of widely different types (3), but no other fruit thus far examined appears to compare with the strawberry in the amount of astringent material present in the very young fruit.

The sugar content of the young fruit varies in amount and in the proportion of reducing sugars and sucrose entering into its make-up. In general, sugars constitute 30 to 40 percent of the soluble solids, but in a few cases the percentage is materially higher. Free reducing sugars in most cases make up 60 to 90 percent of the total sugar, but in a few instances sucrose nearly equals or even exceeds reducing sugars. The occurrence of high sucrose content is quite erratic, a variety in which it is relatively high in one year having very little at a corresponding stage in other years.

Titrateable acidity is in general quite high in the initial samples, its absolute amount fluctuating rather widely from year to year in a given variety. Active-acidity determinations were made only in 1927 and 1928; the results indicate that active acidity is at a minimum in the youngest fruits obtained and undergoes a pronounced rise during subsequent development. Titrateable acidity may undergo a similar but less pronounced increase or may remain more or less stationary throughout the growth of the berry, decreasing slightly in the fully ripe fruit.

The resistance of the fruit to puncture is greatest in the youngest fruits and decreases progressively as development proceeds. In 2 or 3 instances the larger berries taken at the first sampling were slightly firmer than the smaller ones, but in general the smallest fruits obtainable were the firmest.

In summarizing, it may be said that very young fruit, at the time the petals are withering and falling or soon thereafter, is characterized by maximum firmness as measured by resistance to puncture, maximum total solids, maximum insoluble solids, total astringency and nontannin astringency, high acidity, and a rather variable but relatively low total-sugar content consisting predominantly of free reducing sugars with widely varying amounts of sucrose.

SECOND SAMPLING

The fruit collected at the second sampling had entered upon the phase of rapid enlargement on the percentage basis as indicated primarily by increase in volume. The system of grading by size herein employed included in any given sample berries differing by 3 or 4 mm in transverse diameter, the lower limit being the upper limit of the preceding sample. No estimates of average volume of berry were made, but the second-stage fruits averaged 2 to 3 times the volume of the first-stage fruits.

In some instances the larger berries obtained at the initial sampling showed well-defined physical and chemical differences from the smaller fruits taken at the same time; in others, such differences were less distinct. Without exception the fruit obtained at the second sampling, which was made 6 to 10 days after the first, showed distinct and consistent changes in practically every constituent determined.

The most pronounced change was a marked decrease in total solids, which was accompanied in all cases by a decrease in insoluble solids and in nearly all by some reduction in soluble solids. Total and nontannin astringents showed a very marked decrease, proportionally greater than in any other constituent. Total sugars were somewhat variable; in the majority of cases they were slightly lower than at the initial sampling; in the others they were practically stationary or showed a slight increase. In general, those varieties which were exceptionally high in sugar at the first sampling showed rather pronounced decreases; those which were low at that time showed no decrease or an actual gain, with the result that the ratio borne by total sugars to soluble solids was less variable at the second sampling than at the first. Sucrose in the great majority of cases had decreased; in others it remained constant or increased slightly. In no case was there any significant increase or any tendency toward permanent increase of sucrose. Titratable acidity in nearly all cases increased; in the exceptions it was highest in the first sample and declined somewhat at the second sampling. In every case in which hydron concentration determinations were made, the active acidity had very appreciably increased over that of the first sample. In all cases there was a considerable decrease in the resistance of the fruit to puncture.

CHANGES UP TO WHITENING STAGE

To describe in detail the changes that occur from one sampling period to the next for the various stages up to the point at which the fruits begin to lose their chlorophyll and become blushed would involve much repetition. The whole period is one of progressive decrease, initially rather rapid in total solids, insoluble solids, and astringent material. There is a less pronounced decrease, not present in every case, in the soluble solids. Total sugars and titratable acidity are rather variable in behavior, usually increasing slightly but frequently fluctuating irregularly without much net change. The explanation of these facts is clear (3). The period is one of rapid increase in size, due primarily to distention of the cells by absorption of water. The rate of water intake greatly exceeds the rate of formation of structural material. In consequence the percentage of insoluble solids and of total solids present in the fruit progressively decreases as the process of distention with water continues. Meantime, sugars are being transported into the fruit from the leaves at a rate which continually fluctuates with changes in the rate and amount of photosynthetic activity. Some of the incoming sugar is used up in respiration; some is employed in the formation of cell walls and structural material; the remainder accumulates as sugar. Acids are being produced in the fruit as a result of katabolic activities at a rate which fluctuates with changes in the environmental conditions. Whether sugar content shall show an increase in amount from day to day will be determined by the relation between rate of formation and transport of sugar, rate of respiration, and rate of absorption of water. In a period of deficient moisture supply and favorable conditions for photosynthetic activity, the growing fruit would show a rise in sugar content; under the opposite conditions a decrease would occur. In consequence, the composition of the fruit as regards sugar and acid content at any point in the period of rapid

growth is determined by the rate at which absorption of water is occurring in its relation to the rates of photosynthetic and respiratory activity. Because of the progressive accumulation of water the growing fruit decreases progressively in total and insoluble solids throughout the period of rapid growth. Meanwhile the content of sugar and acid fluctuates irregularly by reason of variations in rate of production, which at one time lags behind, at another forges ahead of the accumulation of water. In spite of irregularities due in part to sampling error, there is a clear tendency to an increase in both sugar and acid content with increase in size. Resistance to puncture decreases steadily throughout the period of rapid growth.

CHANGES DURING WHITENING STAGE

The stage of whitening, in which the fruit has practically attained full size and is losing its chlorophyll and beginning to develop a blush, is definitely characterized by a number of chemical changes. The progressive increase in water content which accompanies the period of rapid growth practically ceases and water content becomes nearly or quite stationary at a value that is maximum for the whole life history. Conversely, total solids become stationary at a minimum value before beginning to increase somewhat as the fruit ripens. Titratable acidity, which as a rule has been increasing up to this point, reaches a maximum from which it subsequently declines rather rapidly. Active acidity attains a maximum from which it subsequently decreases somewhat as ripening proceeds. Soluble solids, which have declined somewhat or remained relatively stationary up to this point, show the beginning of an increase which continues up to ripeness. Total sugars, which have previously made slow and irregular gains, increase markedly, and this increase is practically wholly in the form of reducing sugar, the amount of sucrose present remaining stationary or declining somewhat. The total sugars, which during the period of rapid growth make up 50 percent or less of the soluble solids, now compose 60 to 65 percent of the total soluble matter but only 30 to 40 percent of the total solids of the berry. Total astringency, which has been decreasing rather rapidly up to whitening, enters upon a stage of slow and irregular decline, continued decrease in "true tannins" being more or less completely balanced by increase in the nontannin fraction. Insoluble solids, which have declined quite rapidly up to this point but which have hitherto exceeded soluble solids in amount, drop below soluble solids and continue to decrease at a slower rate to full ripeness. Resistance to puncture ranges between 140 and 100 g, or between one-third and one-fourth of that found at the time the petals are falling.

CHANGES DURING RIPENING

The transition from the whitening stage to full ripeness is accompanied by an increase in total solids, ranging in amount from very slight to very considerable, which only very exceptionally fails to occur. This increase is due to a rapid increase in total sugars, which considerably more than compensates for a simultaneous decrease in insoluble solids. While sugars at the whitening stage made up only 60 to 65 percent of the soluble solids and not more than 40 percent of the total solids of the fruit, in the ripe fruit sugars constitute 70 to

80 percent of the soluble solids and 50 percent or more of the total solids. The percentage of sucrose in the total sugars is highly variable in the ripe fruit, as is the case throughout development, and its occurrence is sporadic, the extremes in the same variety ranging between 3 percent and 30 percent of the total sugar present in different years. In general, 80 to 90 percent of the sugar of the ripe fruit consists of reducing sugars. Titratable acidity declines rather rapidly from whitening to full ripeness, then becomes stationary or may rise slightly in the overripe fruit. Active acidity also declines in varying amount. Total astringency declines slowly, increase in the nontannin fraction partially counterbalancing the continued decrease in tannin. Resistance to puncture decreases rapidly, the general average of 100 to 140 g found in the various varieties at whitening stages becoming reduced to 15 to 30 g at firm ripeness and sinking to 5 to 10 g in overripe fruit.

Summarizing the general course of development in the fruit, the earliest stages obtainable are characterized by a high total-solids content consisting chiefly of insoluble materials, a very high content of substances which readily reduce potassium permanganate, variable but relatively low sugar content, moderately high titratable acidity, and low active acidity. The period of rapid increase in volume is one of progressive increase in water content with a resultant decided decline in content of total solids, insoluble solids, and total astringency. In the earlier stages there is a varying and less pronounced decline in soluble solids with which there is generally associated a decrease in the total sugars; later, soluble solids become practically stationary and sugars may show an increase. Titratable acidity increases, active acidity shows a very pronounced increase. At the whitening stage, as the berry passes over from the phase of rapid growth into the ripening processes, water content, titratable acidity, and active acidity attain maximum values. The ripening of the fruit is attended by a marked increase in total sugars and soluble solids; an increase in total solids; and a decrease in total astringency, titratable acidity, active acidity, and insoluble solids. The firmness of the fruit as measured by resistance to puncture decreases throughout the whole period of development and ripening, most rapidly as the fruit passes from whitening to ripeness.

RELATION OF CHANGES IN CHEMICAL CONSTITUENTS TO ONE ANOTHER AND TO RESISTANCE TO PUNCTURE

In the preceding section the changes in chemical composition and in the resistance to puncture occurring in the strawberry during development and ripening have been pointed out and discussed. It now seems advisable to examine in more detail the nature of the relationships which exist among these several changes, and also to ascertain whether these relationships throw any light upon the behavior of the fruit in the course of the various canning and preserving processes.

It is obvious from the preceding discussion that the physical character and chemical composition of the strawberry are functions of its stage of maturity. The growth and development of the berry are influenced by many factors, some of which may affect one phase of its development to one degree and another phase to a different degree, or even in a different manner. Among these factors may be men-

tioned character and composition of soil, fertilizer applications, weather conditions, available soil moisture, prevalence of leaf diseases, closeness of planting, number of berries borne upon the plant, and position of the flower cluster on the plant and of the berry in the flower cluster. It is difficult or impossible to evaluate the effects of these factors upon a given lot of material. For this reason a single series of samples of field-grown fruit may yield results that leave the general relationships between physical and chemical characters in considerable doubt. Since there were available in this study 40 series of samples made up of 278 separate lots of fruit, the material seemed to offer an opportunity to determine more accurately the relationships existing among the several constituents of the berry than would be possible with a smaller number of samples.

Study of the data with reference to maturity of this material has been considerably handicapped by the fact that the exact age of the berries making up any given sample was not known. It was not possible to tag the opening flowers, which would have made it possible to collect samples of any desired age. The approximate age of the fruit was known, however, and it was apparent that its resistance to puncture would give a fairly accurate measure of its degree of maturity. From a practical standpoint the firmness of the fruit is one of its most important characteristics. It therefore seemed desirable to study the relationship between degree of resistance to puncture and the amounts of the various chemical constituents of the fruit. Such a study seemed to be justifiable, for if there is a definite relationship between the resistance of the fruit to puncture and the amounts of the various chemical constituents present, the making of puncture tests would afford an inexpensive, readily obtainable index of the composition of the fruit.

That there is a correlation between the resistance to puncture offered by the fruit and its chemical composition is immediately apparent from an examination of the analytical data. It will be noted that the correlation is not perfect, but just how nearly so it may be is not obvious from inspection. Moreover it is not clear whether the correlation is as high at one stage of development or maturity as at another. In other words, the question arises whether the function is linear or curvilinear. Further, if the data are not in complete agreement, a question arises as to the extent of the variations and as to the reliability of the average values determining the location of the lines or curves representing the relationships.

It is also obvious from an inspection of the data that some degree of correlation exists between the ratio of the insoluble solids to the soluble solids and the chemical composition of the fruit. Precisely the same questions arise in regard to this relationship as to that between firmness and composition, with a like impossibility of obtaining definite answers by inspection of the data. In order to answer these questions as fully as possible, statistical methods were applied to the data, and the results are presented as a series of graphs. These present in part the relationships between resistance to puncture and certain chemical constituents, in part the relationships between the ratio of insoluble to soluble solids and these same constituents.

As the material at hand consisted of samples of 12 varieties of strawberry, an attempt was made at the outset to classify the data

with respect to variety. Thus there were formed 12 groups of samples of widely varying size, which markedly increased the magnitude of the standard error of estimate and resulted in the production of broken and highly irregular curves. The combination of all the data from the 278 samples into one class resulted in the production of very much smoother curves, and such a combination has accordingly been made. It should therefore be remembered that a part of the variation shown in the graphs is to be attributed to varietal differences.

The method employed in making the calculations is one described in detail by Ezekiel (11, pp. 91-97, 111-124), and may be stated briefly as follows. The values of the given chemical constituent under consideration are regarded as the dependent variable y , and are plotted against the ratio of insoluble to soluble solids as the independent variable x . The pairs of values for each of the 278 samples were tabulated and then plotted on coordinate paper on a large scale, each pair of values being represented by a small circle. The line representing the average for all these points was then determined freehand, as follows: The values were divided into groups along the x axis, and the values of these group averages were each represented by a point; these points were connected by a line which was generally somewhat irregular. In order to eliminate these irregularities larger group averages were made or the groups were combined so as to establish intermediate points. Finally, a smooth curve was drawn in such a position that the deviations of the points on either side of the line appeared to be equal. This line represents the regression of the dependent variable upon the independent variable, and gives a complete picture of most of the facts in regard to the relationship between the two variables. The vertical distance of each point from the line of regression was read directly from the graph. The value of each of these deviations was recorded with its proper sign, and added algebraically to the actual value of the dependent variable to which it corresponded. A set of estimated values was thus obtained. The standard deviation of these estimated values, divided by the standard deviation of the actual values, gives the coefficient of correlation. Since certain corrections have to be made it is necessary to employ formulas which take these into account. The symbols and formulas and the general method employed are as stated by Ezekiel (11, pp. 399-403). The formulas are numbered as in Ezekiel's list.

x =independent variable.

y =dependent variable (actual value).

y' =estimated values of the dependent variable.

z =residual or difference between actual and estimated values
of the dependent variable.

M =mean.

σ =standard deviation.

S =standard error of estimate.

r =coefficient of correlation.

ρ =index of correlation.

$$\sigma_x = \sqrt{\frac{\sum(X^2)}{n} - n(M_x)^2} \quad (5)$$

$$r_{yx} = \frac{\sigma_y'}{\sigma_y} \quad (21)$$

$$\bar{r}_{yx}^2 = 1 - (1 - r^2) \left(\frac{n-1}{n-2} \right) \quad (25)$$

$$\rho_{yx} = \frac{\sigma_{y''}}{\sigma_y} \quad (22)$$

$$\bar{\rho}_y^2 = 1 - (1 - \rho^2) \left(\frac{n-1}{n-m} \right) \quad (26)$$

$$\bar{\rho}_{yx}^2 = 1 - \left(\frac{\sigma_{z''}^2}{\sigma_y^2} \right) \left(\frac{n-1}{n-m} \right) \quad (29)$$

$$\bar{S}_{yx}^2 = \frac{n\sigma_z^2}{n-2} \quad (23)$$

$$\bar{S}_{y-f(x)}^2 = \frac{n\sigma_{z''}^2}{n-m} \quad (24)$$

$$\sigma r_{yx} = \frac{1 - r^2}{\sqrt{n-2}} \quad (71)$$

$$\sigma \rho_{yx} = \frac{1 - \rho^2}{\sqrt{n-m}} \quad (72)$$

The results of the calculations are summarized in table 2; the various curves to which they relate are shown in figures 1 to 12.

TABLE 2.—Coefficients and indices of correlation and standard errors for the correlations between resistance to puncture, ratio of insoluble to soluble solids, and various chemical constituents of the strawberry during development and ripening

Independent variable	Dependent variable	Coefficient or index of correlation	Standard error of the coefficient or index of correlation	Coefficient of determination	Standard error of estimate
Resistance to puncture.	Ratio of insoluble to soluble solids.	+0.917	±0.009	0.841	0.207
Do	Percentage of insoluble solids	+ .882	± .014	.778	1.125
Do	Percentage of soluble solids	+ .641	± .033	.412	.805
Do	Percentage of total solids	+ .754	± .026	.568	1.455
Ratio of insoluble to soluble solids.	do	+ .884	± .013	.781	1.357
Resistance to puncture.	Percentage of total sugars	-.817	± .019	.668	.741
Ratio of insoluble to soluble solids.	do	-.871	± .014	.759	.584
Resistance to puncture.	Percentage of titratable acidity	+ .494	± .045	.245	.223
Ratio of insoluble to soluble solids.	do	+ .547	± .041	.299	.204
Resistance to puncture.	Percentage of total astringency	+ .926	± .008	.857	.459
Ratio of insoluble to soluble solids.	do	+ .710	± .028	.504	.685
Percentage total sugar.	Percentage of titratable acidity	-.290	± .54	.064	.247

¹ Index. The curve is in part positive, in part negative.

RATIO OF INSOLUBLE TO SOLUBLE SOLIDS IN RELATION TO RESISTANCE TO PUNCTURE AND THEIR SIGNIFICANCE AS MEASURES OF MATURITY

Since the exact age of the fruit comprising a sample was not known, it was impossible to determine exactly how well the degree of resistance to puncture measured the degree of maturity, for neither set of values could be checked against the age of the sample. As a means of checking the results of the puncture test, which is a physical method of estimating maturity, it was desirable to employ another and entirely different method, chemical in character, for estimating stage of matur-

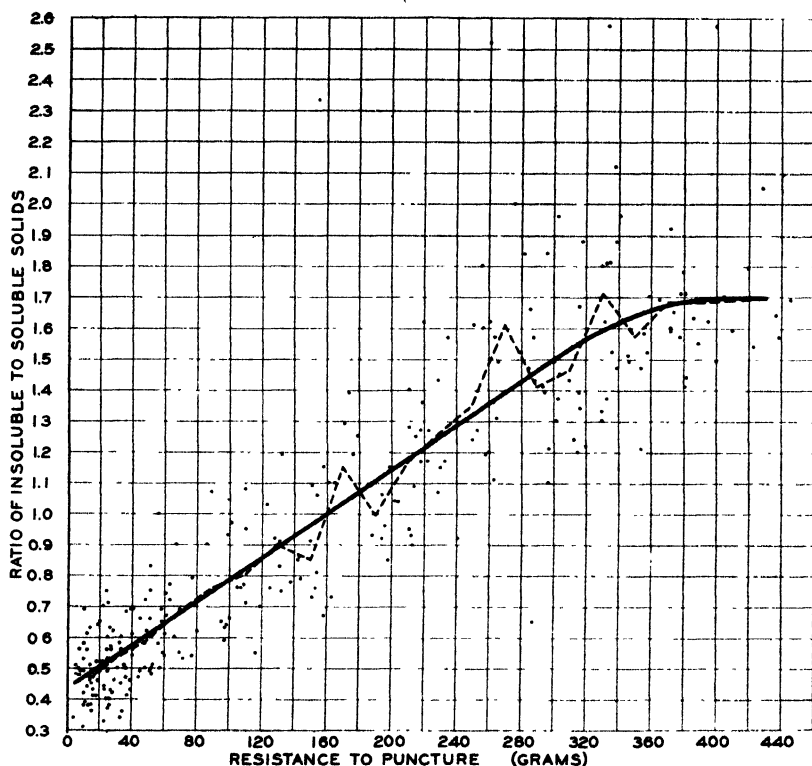


FIGURE 1 — Relationship between ratio of insoluble to soluble solids and resistance to puncture during development of the strawberry. Plotted from table 1. Includes all values for all varieties for four seasons. Broken lines indicate position of the group averages; the heavy line is a smoothed curve. The coefficient of correlation is 0.917 and the standard error of estimate 0.207.

ity. An examination of the analytical data in some detail appeared to indicate that the ratio of insoluble solids to the soluble solids of the fruit is also a good measure of the stage of maturity; it was therefore chosen to serve as a check upon the results obtained by the use of the puncture test.

In order to ascertain the degree of relationship between these two measures of maturity, values for the ratio of insoluble to soluble solids were plotted against values for the puncture test, the line of regression being determined as drawn freehand in the manner already described. The result is shown in figure 1. It may be pointed out that for low values of both ratio and puncture test the fruit was fully

ripe; for high values of both measures the fruit was very immature. The line of regression is very nearly a straight line, but the data seem to justify a slight curve downward of the end of the line as extremely high values of the puncture test are reached. This shows that, on an average, changes in the values for the ratio of insoluble to soluble solids are directly proportional to changes in the resistance of the fruit to puncture, except for a small group of very high values. It may be noted that the points representing the individual pairs of values do not fall exactly upon a line but form a rather narrow band, the center of which is a line representing the average of all values.

From the amount and character of the scatter about the line of regression it is evident that in individual cases the change in one of the measures employed is not accompanied by a corresponding change in the other. This of course means that if estimates of the degree of maturity were made for each of the samples in hand by the two methods of estimation separately the results in the individual cases would differ somewhat. While some part of the scatter, especially the widely aberrant values occurring here and there in the graph, are undoubtedly due to errors in making the analytical determinations, it is believed that environmental conditions may have been such as to affect one of these criteria of maturity without affecting the other to the same degree.

The coefficient of correlation was determined and found to be 0.917, which indicates a very good agreement between the two measures of maturity. The coefficient of determination is 0.84. The standard error of estimate (0.207) shows that in 1 out of 3 cases the actual values of the ratio of insoluble to soluble solids will fall below or exceed the estimated values by as much as 0.207 percent. The reliability of the line of regression is not the same throughout its length, but decreases when the values are very high; in other words, with very young samples of fruit.

INSOLUBLE SOLIDS IN RELATION TO RESISTANCE TO PUNCTURE

In figure 2 the values for content of insoluble solids are plotted against the values for the puncture test. The points, with relatively few exceptions, fall closely about a locus which appears to be a straight line. For low values of the puncture test the content of insoluble solids is low; for high values it is high. The correlation is consequently positive and a coefficient of 0.882 shows a rather high degree of correlation. From the coefficient of determination it is seen that 78 percent of the total variation in the resistance to puncture of the berries is accounted for by differences in insoluble solids, leaving only 22 percent to be explained by all other causes, including variable differences. The standard error of estimate (1.125) indicates that on an average two-thirds of the values for insoluble solids will fall within the limits of 1.125 percent above or below the line of regression. The reliability of the line, however, is not so great where the values are high as where they are low, as it evidenced by the increase of scatter in the high values (fig. 2). Since high values of the puncture test are indicative of immaturity, it is evident that the percentage of insoluble solids in the fruit is inversely proportional to the degree of maturity of the berry.

SOLUBLE SOLIDS IN RELATION TO RESISTANCE TO PUNCTURE

In figure 3 the percentages of soluble solids in the various samples are plotted against the corresponding values of the puncture test.

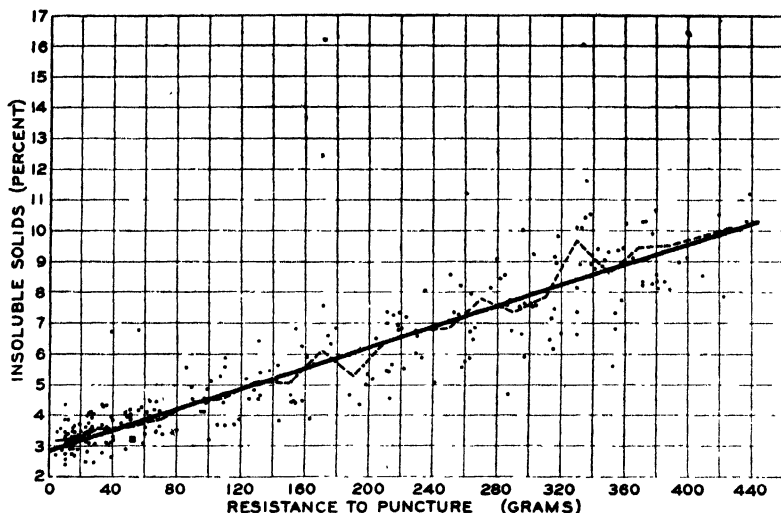


FIGURE 2.—Relationship between percentage of insoluble solids and resistance to puncture during the development of the strawberry, plotted from the data of table 1 and including all values for all the varieties for the 4 years. Broken lines indicate the position of the group averages; the heavy line is a smoothed curve. The coefficient of correlation is 0.882 and the standard error of estimate 1.125.

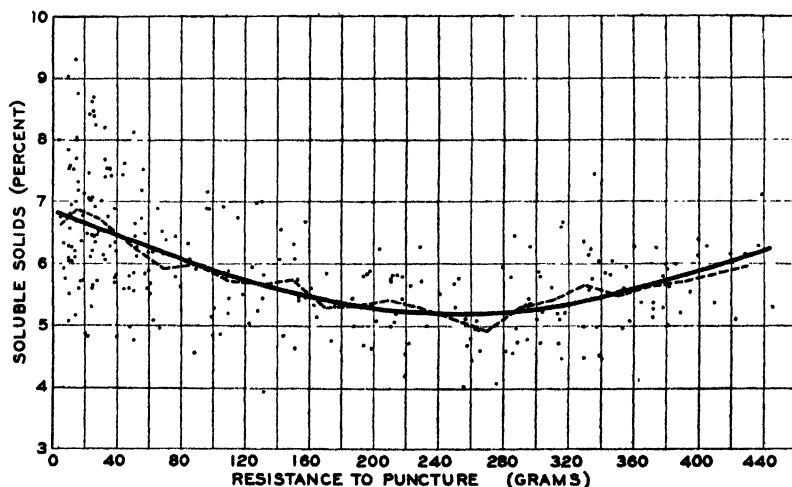


FIGURE 3.—Relationship between percentage of soluble solids and resistance to puncture during the development of the strawberry, plotted from data of table 1. The index of correlation is 0.641 and the standard error of estimate 0.808.

The points representing the various samples fall in a broad band about a locus which appears to be a pronounced curve, the scatter about the mean increasing very materially as low values for the

resistance to puncture are reached. The form of the curve is the resultant of several chemical changes differing in direction but occurring concurrently during the development of the fruit. The three groups of substances mainly concerned are sugars, acids, and tannins. Tannins and acids are high in the immature fruit and decrease with advancing maturity; sugars are low in the young fruit and rise rapidly as maturity is reached. The resultant line of regression is a curve which indicates a negative correlation where the values of the puncture test are low and a positive correlation where the puncture-test values are high. In other words, the correlation of soluble solids with puncture test is positive during the period just subsequent to flowering but becomes negative during the ripening period. The index of correlation of 0.641 is very considerably less than that found for insoluble solids. The coefficient of determination shows that 41 percent of the

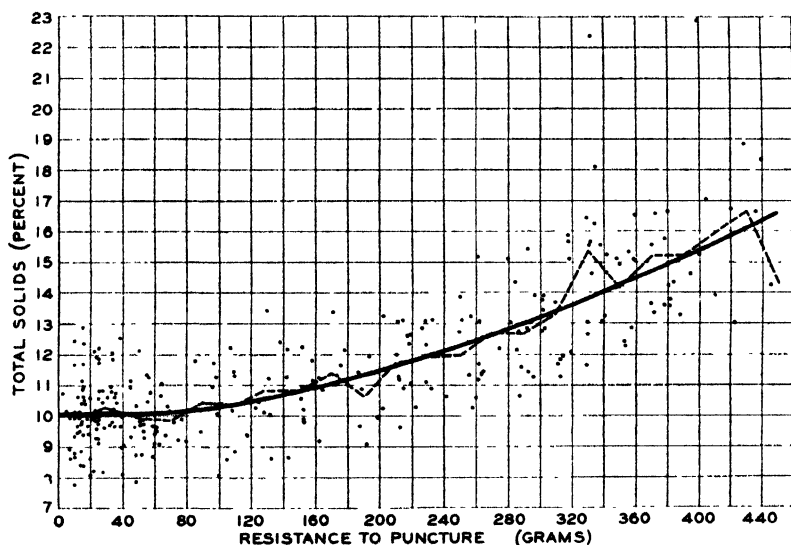


FIGURE 4 -- Relationship between percentage of total solids and resistance to puncture during development and ripening of the strawberry, plotted from the data of table 1. The coefficient of correlation is 0.754 and the standard error of estimate 1.455.

total variation in resistance to puncture may be accounted for by the differences in soluble solids, leaving 59 percent to be attributed to all other causes. This is a considerably larger percentage of unexplained variation than was the case with the insoluble solids. It is due not so much to the greater variation in the soluble solids as to a lack of a proportional change in soluble solids with change in resistance to puncture. The standard error of estimate (0.805) is numerically lower for soluble solids than for insoluble solids.

TOTAL SOLIDS IN RELATION TO RESISTANCE TO PUNCTURE

The results obtained when total solids of the fruit were plotted against resistance to puncture are presented in figure 4. The points are distributed over a broad band and the line of averages forms a rather pronounced curve. For puncture tests ranging from 5 to 80 g there is on an average little change in the percentage of total solids,

although the scatter is somewhat wider in this portion of the graph than elsewhere. This indicates that there is no correlation for the values in this range. For values of the puncture test above 80 g the percentage of total solids progressively rises; that is, resistance to puncture and percentage of total solids increase together. A change of a given magnitude in resistance to puncture is not accompanied by the same amount of change in the percentage of solids for all values of the puncture test. Despite the lack of correlation over part of the range of values, the coefficient of correlation is 0.754. This is a fair degree of correlation and the index of determination shows that 57 percent of the total variation is accounted for, leaving 43 percent as due to all other causes. The standard error of estimate (1.455) shows that two-thirds of the values lie within limits of 1.455 percent above or below the regression line.

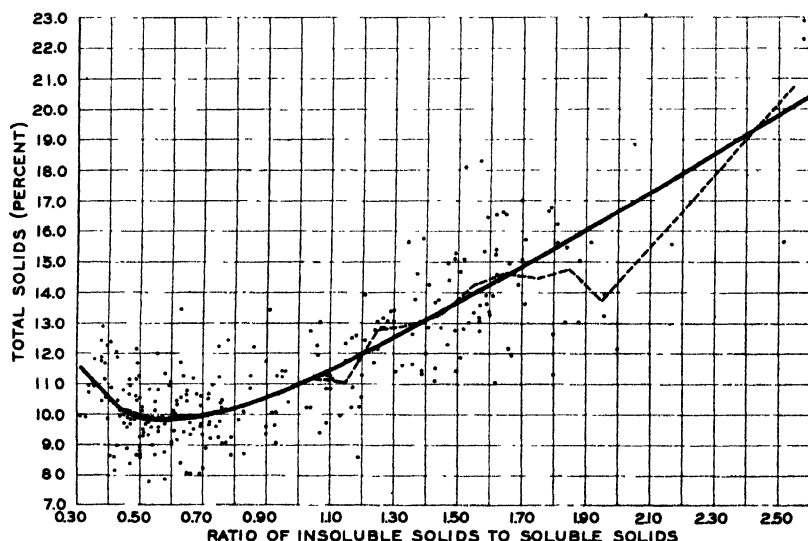


FIGURE 5.—Relationship between percentage of total solids and the ratio of insoluble to soluble solids during the development and ripening of the strawberry, plotted from the data of table 1. The index of correlation is 0.894 and the standard error of estimate 1.357.

The form of the curve is due very largely to changes occurring in the developmental period of the fruit. The curve is the resultant of the joint effect of concurrent changes in a number of substances, some of which are increasing while others are decreasing.

In view of the large standard error of estimate and the large percentage of the variation left unexplained, it seemed worth while to plot total solids against the ratio of insoluble to soluble solids in order to ascertain how closely the results would check with the picture of relations just given, and this was accordingly done.

TOTAL SOLIDS IN RELATION TO RATIO OF INSOLUBLE TO SOLUBLE SOLIDS

Total solids have been plotted against the values for the ratio of insoluble to soluble solids in figure 5. For low values of the ratio of insoluble to soluble solids the total solids are low; and for high values the total solids are also high; that is, for high values of the ratio of

insoluble to soluble solids and for high values of the resistance to puncture, the fruits are very immature, and for low values they are mature. Intermediate values represent intermediate stages of maturity. The points representing the various pairs of values for total solids and the ratio of insoluble to soluble solids are rather symmetrically distributed around a line which is a pronounced curve. In general it is quite similar to the puncture-test curve of figure 4, but in one respect the two curves are quite different. The curve for solids plotted against resistance to puncture was quite flat for values of resistance to puncture ranging from 5 to 80 g, that is, in that portion of the curve representing the ripening period. In the corresponding portion of the curve for solids plotted against the ratio of insoluble to soluble solids, the curve first turns sharply downward, indicating a negative correlation, then changes direction, becoming nearly flat, after which it rises like the curve of figure 4 throughout the remainder of its length. Its direction in this latter portion indicates that there is a strong positive correlation with the ratio of insoluble to soluble solids, as was the case with the like portion of the curve for solids plotted against resistance to puncture. The extreme opposite end of this curve is negative, while the corresponding portion of the curve for solids plotted against resistance to puncture shows no correlation during growth and up to the onset of ripening, indicating that the berry at this period has its highest moisture content, after which solids rise again as the fruit becomes fully ripe. These facts are not shown by the curve for solids in relation to resistance to puncture. A detailed study of the two curves in connection with notes made at the time of picking as to the approximate age, color, and size of the fruit leads the writers to the conviction that the curve for relationship of total solids to the ratio of insoluble to soluble solids gives a truer picture of the changes in total solids during the development and ripening of the fruit than does the curve for relation of total solids to resistance to puncture. This conviction is reinforced by the fact that the index of correlation (0.884) is higher and the standard error of estimate (1.357) lower than for the curve for solids plotted against resistance to puncture; also that a considerably larger proportion of the variation, 78 percent as against 57 percent, is accounted for. All the facts point to the conclusion that while there is in general good agreement for the curves, that of figure 5 is more reliable than that of figure 4, particularly in that portion representing the ripening period of the fruit.

TOTAL SUGARS IN RELATION TO RESISTANCE TO PUNCTURE

In figure 6 the percentages of total sugar have been plotted against resistance of the fruit to pressure. For low values of the puncture test total sugars are high; for high values they are low. The correlation between sugars and resistance to puncture is therefore negative. The values are distributed about a line which is a pronounced curve for part of its length, becoming a horizontal line in its right-hand portion. A line of regression of this character shows that a unit change in the value of the puncture test is not accompanied by the same amount of change in percentage of sugar at all stages throughout the development. Such a change in puncture test when the resistance of the fruit to puncture is low, that is, after the ripening process has set in, is accompanied by a much larger change in the percentage

of sugar than is the case for high values of the puncture test, that is, in immature fruit. An index of correlation of 0.817 shows a fairly good correlation, and the index of determination indicates that 67 percent of the variation is accounted for by differences in the puncture test, leaving 33 percent to be explained by all other factors. As the varieties show somewhat wider varietal differences in sugar content than in other constituents, a portion of this unexplained variation is unquestionably due to varietal differences, but variations in environmental conditions and errors of determination also have an influence. The standard error of estimate is 0.741 indicating that on an average two-thirds of the values lie between the limits 0.741 percent above and 0.741 percent below the line of regression. The deviation of the points from the line becomes somewhat greater during the period of

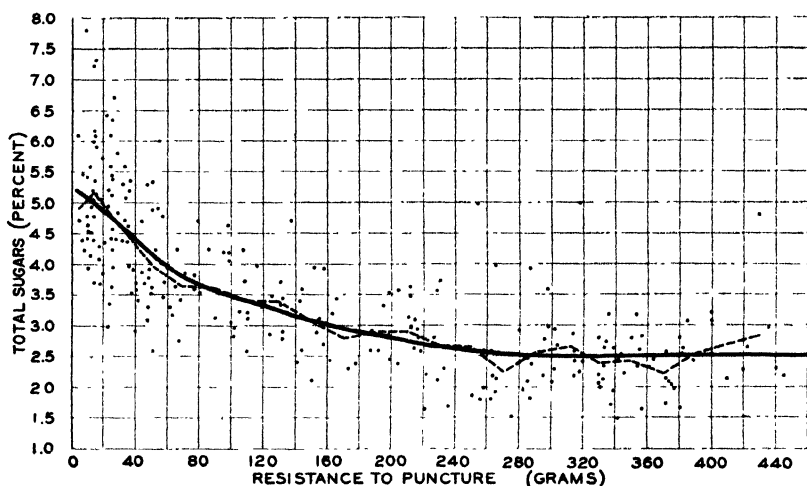


FIGURE 6.—Relationship between percentage of total sugars and resistance to puncture during the development and ripening of the strawberry, plotted from the data of table 1. The index of correlation is 0.817 and the standard error of estimate 0.741.

ripening, that is, as the values for the puncture test become low. This may mean that the puncture test is less reliable as a measure of maturity as the berries approach full ripeness than when they are still quite firm, or it may mean that the berries are more influenced by environmental factors at this stage of maturity than at earlier stages.

TOTAL SUGARS IN RELATION TO RATIO OF INSOLUBLE TO SOLUBLE SOLIDS

In view of the uncertainties just suggested it seemed advisable to plot total sugars against the ratio of insoluble to soluble solids (fig. 7). It will be noted that the curve, while in general similar to the curve showing relation of total sugars to resistance to puncture, is more pronounced. The index of correlation (0.871) is higher and the error of estimate (0.584) is lower, indicating that the curve is somewhat more reliable than that of figure 5, especially in the period of ripening of the fruit. From this it may be inferred that the pressure test becomes a somewhat less trustworthy index of stage of maturity as the fruit enters the period of ripening than it is in the case of immature and growing fruit.

TITRATABLE ACIDITY IN RELATION TO RESISTANCE TO PUNCTURE

Figure 8 presents the results obtained when the percentages of titratable acidity of the various samples are plotted against their

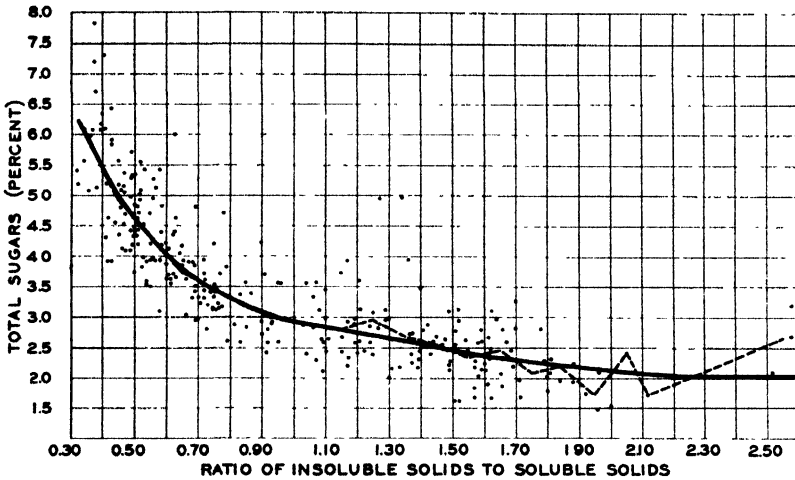


FIGURE 7.—Relationship between percentage of total sugars and ratio of insoluble to soluble solids during the period of development and ripening of the strawberry, plotted from data of table 1. The index of correlation is 0.871 and the standard error of estimate 0.584.

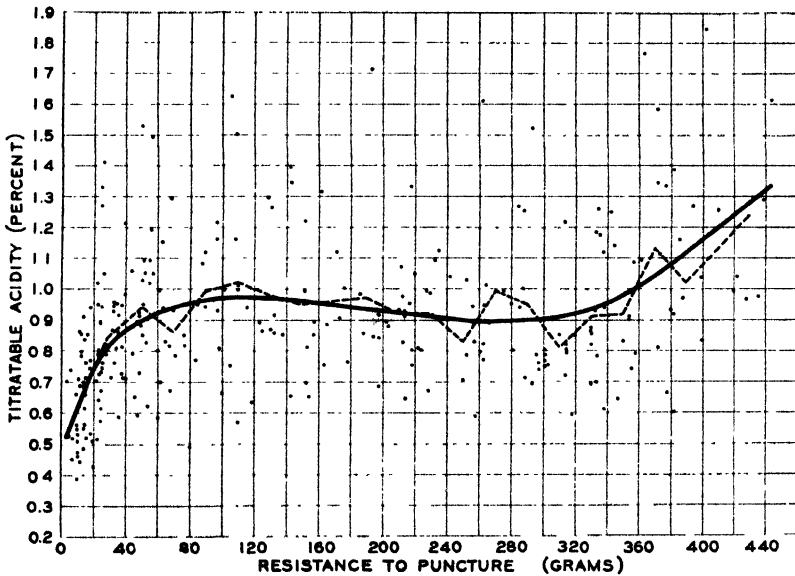


FIGURE 8.—Relationship between percentage of titratable acidity and resistance to puncture during development and ripening of the strawberry, plotted from data of table 1. The index of correlation is 0.494, and the standard error of estimate 0.223.

resistance to puncture. It is obvious that the titratable acidity to a considerable degree varies independently of the resistance to puncture. For low values of the puncture test the acidity is generally

low; for high values it is higher. The curve first rises sharply with increase in resistance to puncture in the region between 5 and 80 g, then becomes nearly horizontal, finally rising again with values of the puncture test ranging from 350 to 450 g. While the final upward turn of the curve follows the group averages very closely, its form must be regarded as somewhat questionable in view of the wide scatter which is apparent. If the final upward portion of the curve be omitted as questionable, it is evident from the form of the curve that there is a good positive correlation between resistance to puncture and titratable acidity throughout the ripening period, with very little or no correlation throughout the period beginning a few days after blooming and extending up to the onset of ripening.

The index of correlation is 0.494, which is quite low. The coefficient of determination indicates that 24.5 percent of the total variation in

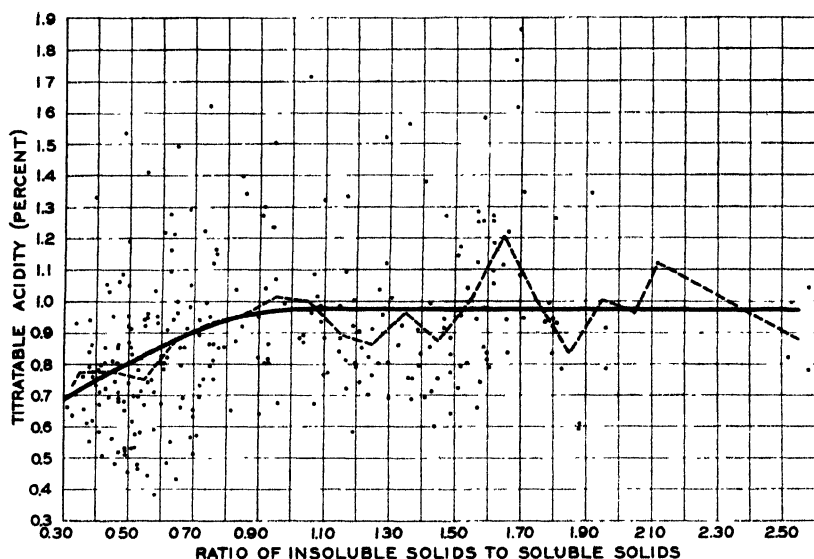


FIGURE 9.—Relationship between percentage of titratable acidity and ratio of insoluble to soluble solids during the development and ripening of the strawberry, plotted from data of table 1. The index of correlation is 0.547 and the standard error of estimate 0.204.

acidity is accounted for by variation in resistance to puncture, leaving 74.5 percent to be attributed to other causes. The standard error of estimate (0.223) indicates that one-third of all the values fall outside the limits of 0.223 percent above or below the line of regression. In terms of the total acidity of the fruit, this is a very considerable variation.

TITRATABLE ACIDITY IN RELATION TO RATIO OF INSOLUBLE TO SOLUBLE SOLIDS

Because of the low correlation between titratable acidity and resistance to puncture, the correlation between titratable acidity and the ratio of insoluble to soluble solids was determined. The results are presented in figure 9.

Throughout most of its extent the curve is similar to that of figure 8 for titratable acidity in relation to resistance to puncture. The chief

difference is in that portion of the curve derived from the very young fruits, which shows no such upward turn as does the curve for acidity in relation to resistance to puncture. The form of the curve indicates no correlation in the case of fruits that are immature, with a fairly good correlation in the case of fruits that have entered upon the ripening period. The index of correlation is 0.547, as compared with 0.494 for the acidity-puncture curve, and the standard error of estimate (0.204) is somewhat lower than for that curve. While both curves indicate little or no correlation for the period prior to the onset of ripening, both indicate a good positive correlation for the ripening period, the values for acidity in relation to ratio of insoluble to soluble solids indicating that this curve is somewhat more reliable

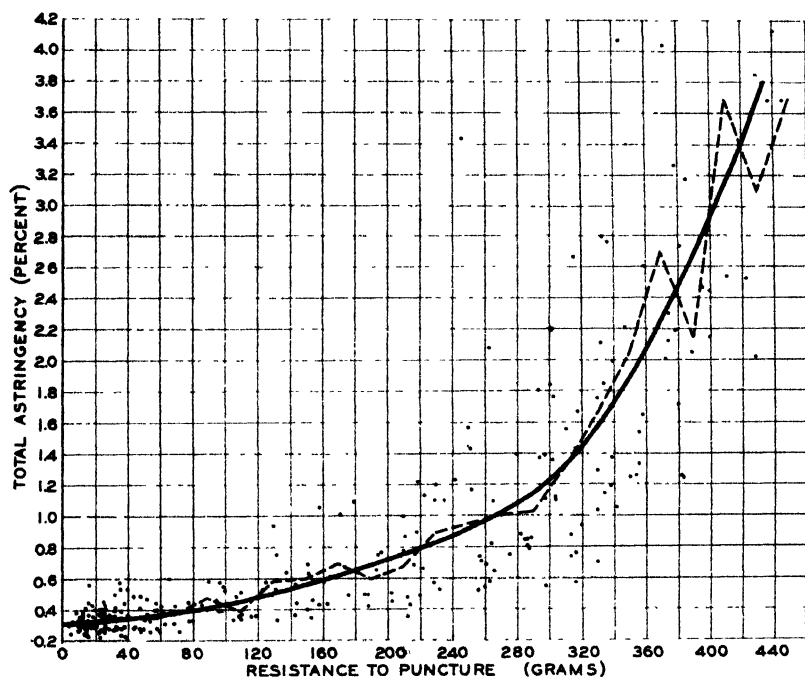


FIGURE 10.-- Relationship between percentage of total astringency and resistance to puncture during development and ripening in the strawberry, plotted from data of table 1. The index of correlation is 0.926 and the standard error of estimate 0.459.

than the curve showing the relationship of acidity to resistance to puncture.

TOTAL ASTRINGENCY IN RELATION TO RESISTANCE TO PUNCTURE

Figure 10 presents the results when total astringency is plotted against resistance to puncture. The points fall rather closely about a locus which appears to be a very striking curve. The form of the curve shows that the regression of total astringency on resistance to puncture is constantly changing throughout the development and ripening of the fruit. For low values of the total astringency the resistance to puncture is low; for high values of total astringency the resistance to puncture is high; and there is a very good positive corre-

lation throughout the entire range of values. The index of correlation is 0.926. The index of determination shows that 86 percent of the total variation in astringency may be accounted for by the variation in resistance to puncture. The standard error of estimate (0.459) indicates that only one-third of the values will fall more than 0.459 percent above or below the indicated line of regression. The line is not equally reliable throughout its length, however. It is much more trustworthy in the period of later development and ripening than in the period of early development, as is evidenced by the progressive increase in scatter of the plotted values as the values of the puncture test increase. The reason for this is not clear. It may be due in considerable part to the fact that the method of determination of total

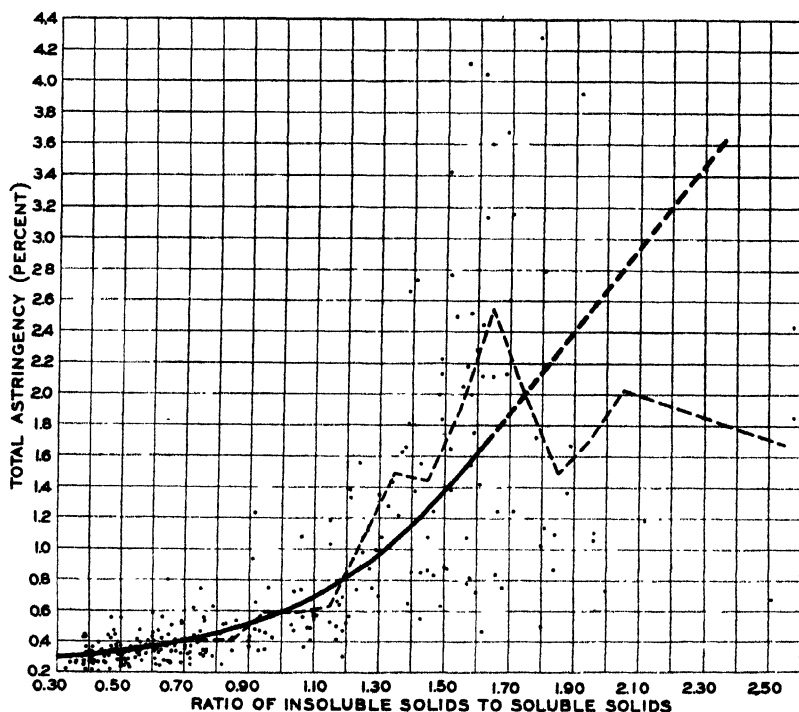


FIGURE 11.—Relationship between percentage of total astringency and ratio of insoluble to soluble solids during development and ripening in the strawberry, plotted from data of table 1. The index of correlation is 0.710 and the standard error of estimate 0.685. The portion of the curve represented by a broken line is questionable because of the relatively small number of samples employed in its determination.

astringency determines a number of related substances that differ in their reducing power for potassium permanganate and that vary independently in amount as the development of the fruit proceeds, which is known to be the case. In part it may be attributed to sampling error and to any or all of the factors already mentioned.

TOTAL ASTRINGENCY IN RELATION TO RATIO OF INSOLUBLE TO SOLUBLE SOLIDS

For comparison with the last curve, the total astringency was plotted against the ratio of insoluble to soluble solids. The results are presented in figure 11.

The form of the curve is very similar to that of the astringency-puncture curve insofar as that portion derived from the later period of development and ripening is concerned. In the case of very immature fruits the number of fruits is small, the scatter of individual fruits is very great, and the line joining group averages is consequently excessively irregular. This may be taken to indicate that the ratio of insoluble to soluble solids as a measure of maturity is not as reliable for very immature fruits as for fruits approaching maturity. There is therefore some question as to whether the form of this portion of the curve, as determined from the number of samples in hand, is what it would be had a larger number of fruits been used. The portion of the smoothed curve which is regarded as questionable is represented on the graph (fig. 11) as a broken line. In the ripening period it is about as good an index of maturity as the puncture test. For the line as a whole the index of correlation is 0.710 and the standard error of estimate is 0.685, with a coefficient of determination of 0.504. These values indicate that the puncture test has considerably greater reliability as a measure of the astringency content than has the ratio of insoluble to soluble solids. The general similarity of the two curves makes the general course of the changes in total astringency fairly clear.

RELATIONSHIP BETWEEN SUGAR CONTENT AND ACID CONTENT

Since the relationship between percentage of acid and percentage of sugar present is a very important factor in determining the palatability of the fruit, it seemed advisable to determine the general relationship between the two constituents in this assemblage of material. In figure 12 the percentage of total titratable acidity is plotted against percentage of total sugars. The scatter of the individual points is very wide, indicating that variations in acid content to a very large extent are independent of the percentage of sugar present. There is a general tendency for titratable acidity to be low when sugars are high, that is, in berries at or nearing maturity, and for acidity to be high when sugars are low, that is, in immature or young fruit. The line which represents this relationship is very nearly straight for most of its length, indicating absence of correlation, but rises somewhat in the portion representing very young samples to indicate a low negative correlation. The absence of any pronounced correlation must mean that some factor not accounted for affects one of the constituents without affecting the other. The fact that environmental conditions, which control the rate of accumulation of sugar through controlling the opportunities for photosynthesis, do not have any comparable controlling effect upon the rate of formation or destruction of acids suggests itself as an explanation of the form of the curve.

DISCUSSION OF GRAPHS

Despite the fact that the curves presented in figures 1 to 12 are not rate curves with respect to time, they tend to confirm the general conclusions with respect to the chronological sequence of events in the development of the berry which were stated in the preceding section. The mathematical treatment substantially reinforces most of the statements there made while at the same time it emphasizes the high degree of variability encountered in a large number of samples of different varieties taken over a period of years.

The question as to which of the two methods of measuring stage of maturity that have been applied to the material is the more reliable cannot be answered definitely, since the exact age of the samples was not known. A fairly conclusive answer to the question can be derived from a comparative study of the results of application of the two methods to the data. In all cases in which this has been done, with one exception, the ratio of insoluble to soluble solids appears to be the more reliable of the two. This method, which may be termed a chemical measure of maturity, is least satisfactory when applied to very immature fruits. The resistance of the fruit to puncture, which

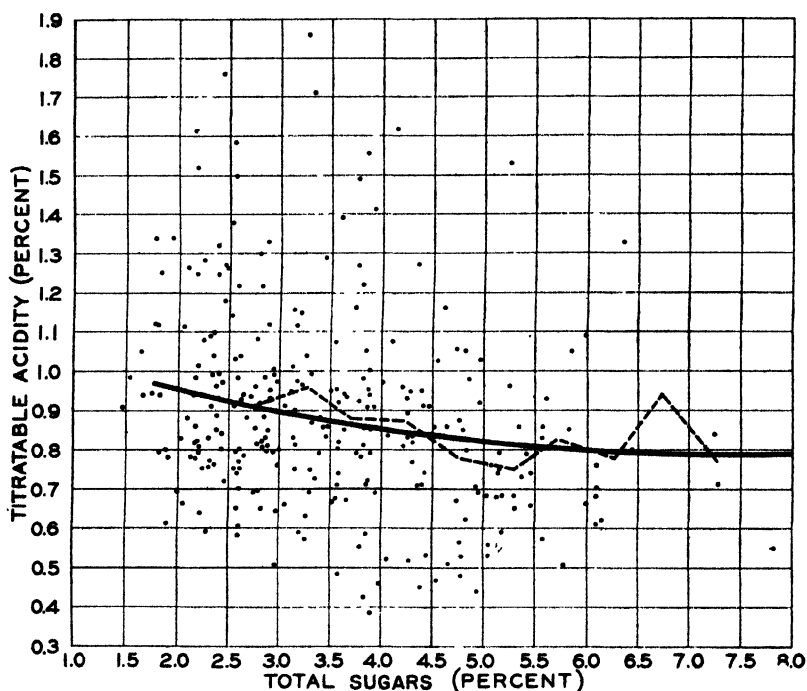


FIGURE 12.—Relationship between percentage of titratable acidity and percentage of total sugars during development and ripening of the strawberry, plotted from data of table 1. The coefficient of correlation is 0.290 and the standard error of estimate 0.247.

is a physical measure of maturity, is very satisfactory for immature fruits, but becomes less reliable as the fruit enters upon the period of ripening. This is true for the particular puncture tester here used; it is, of course, not known what the results would be with other types of testers.

A probable explanation of the failure of agreement between the two measures of maturity in the ripening stage is that environmental conditions at this stage in the life of the fruit exert a considerably greater influence upon the firmness of the berries than upon the relation between insoluble and soluble solids.

PHYSIOLOGICAL CHARACTERISTICS OF INDIVIDUAL VARIETIES

By comparative study of the ripe samples of the several varieties it should be possible to ascertain the degree to which any given variety possesses a definite chemical individuality.

Such a comparison is difficult for the reason that the composition of the ripe fruit of a variety varies considerably from year to year and also from beginning to end of a single crop season, in response to changes in climatic conditions, load of fruit upon the plants, and other varying factors. Since measurements of these factors which would permit any accurate evaluation of their effects could not be made in connection with this study, it can only be assumed that all the varieties were influenced thereby in the same direction and to the same degree and that the averaging together of all the ripe samples of a given variety should give mean values for that variety which are fairly comparable with those obtained in like manner for other varieties. Such average values have been computed and are presented in table 3. The results present a chemical definition or description of each of the varieties in terms of its content of eight constituents.

In order to facilitate comparison of one variety with another, some standard of comparison is desirable. Such a standard, or norm, of entirely arbitrary character has been obtained by averaging together all the data for the ripe samples of all varieties. This treatment permits comparison of any given variety with respect to any given constituent with any other variety or with the average for all the varieties. It does not give any clear concept of the variety as a chemical entity nor does it permit satisfactory comparison of varieties as chemical individuals with one another. In order to make such comparisons it is necessary to treat the data of table 3 in such a manner that the several varieties are presented as entities. Such a method of treatment has been adopted in figures 13 and 14, in which the chemical composition of each of the varieties is represented by a line. The scales upon which the various constituents are plotted were so chosen that the mean values obtained by averaging all varieties fall in a horizontal line across the graph. The composition of each variety was then plotted, and the points on the several scales connected by a line which rises above or falls below the line of general average according as the content of a particular constituent is greater or less than the general average.

TABLE 3.—Average composition of all ripe samples of each of 12 strawberry varieties

Variety	Total solids	Soluble solids	Sugars	Insoluble solids	Titratable acidity	Total astringency	Tannin	Nontannin
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Aroma.....	9.77	6.48	4.63	3.29	0.593	0.365	0.217	0.148
Chesapeake.....	10.93	7.61	5.88	3.33	.745	.303	.162	.141
Dunlap.....	10.11	6.33	4.27	3.79	.736	.321	.183	.138
Gandy.....	11.97	8.35	6.34	3.62	.972	.384	.210	.174
Howard 17.....	9.95	6.96	5.04	3.00	.780	.289	.177	.112
Klondike.....	10.63	7.37	4.99	3.26	.969	.378	.238	.140
Missionary.....	10.89	7.33	5.26	3.56	.772	.389	.217	.172
New York.....	11.66	8.46	6.53	3.19	.656	.275	.114	.161
Parsons.....	9.29	6.16	4.49	3.13	.689	.354	.203	.151
Portia.....	9.08	5.96	4.35	3.12	.586	.280	.138	.142
Progressive.....	9.73	6.32	4.68	3.41	.628	.272	.162	.120
Sample.....	9.38	6.27	4.55	3.11	.613	.362	.195	.167
Average.....	10.16	6.87	5.00	3.29	.705	.319	.178	.141

As a further means of comparing varieties with one another, the ratios between certain constituents, namely, between acid and astringency, acid and sugar, acid and total solids, astringency and total solids, and total solids and sugar, have been calculated for each of the varieties and also for the average of all varieties. These ratios, which are based upon the data of table 3, are brought together in table 4. The mean ratios for all samples form a second standard of reference for facilitating comparisons between varieties.

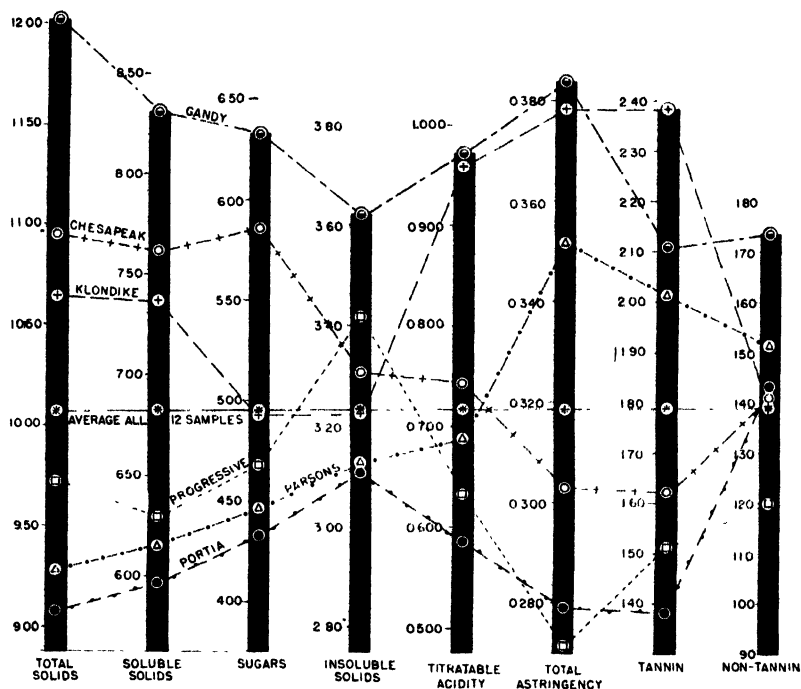


FIGURE 13.—Mean values for total solids, soluble solids, insoluble solids, sugars, titratable acidity, total astringency, tannin astringency, and nontannin astringency in ripe samples of the Gandy, Chesapeake, Klondike, Parsons, Progressive, and Portia varieties, plotted against mean values for the same constituents in all ripe samples of all varieties.

At the same time that the individual characteristics of the several varieties are discussed an attempt will be made to combine the varieties into groups on the basis of similarity of composition. The basis of such grouping is not likeness in absolute amounts of the various constituents, as solids or sugar, in the varieties concerned, but similarity in the relationships existing between the several constituents of the fruit as indicated by the ratios of table 4. Employment of these ratios permits comparison without reference to the absolute amounts of the substances concerned.

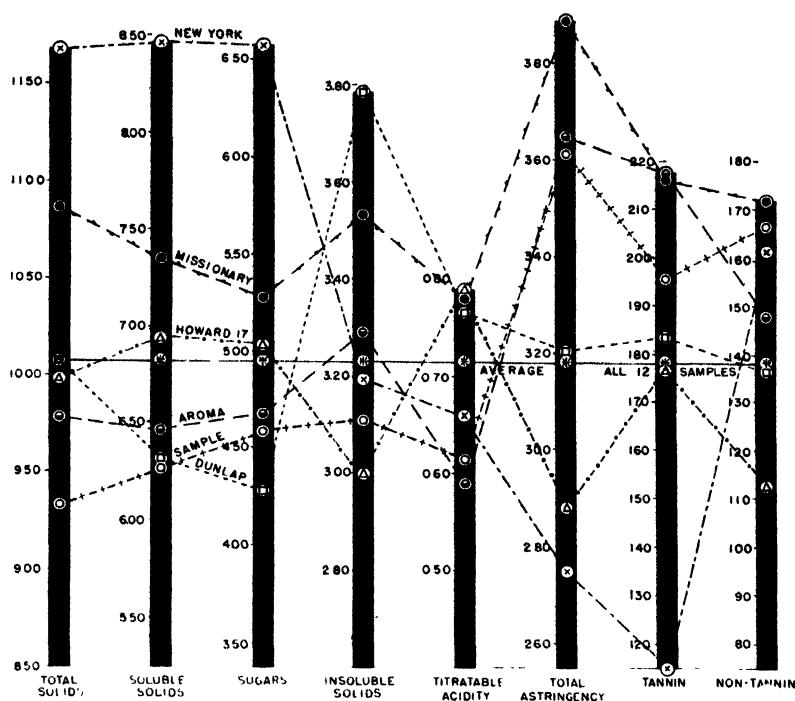


FIGURE 14 Mean values for total solids, soluble solids, insoluble solids, sugars, titratable acidity, total astringency, tannin astringency, and nontannin astringency in ripe samples of the New York, Missionary, Dunlap, Howard 17 (Premier), Sample, and Aroma varieties, plotted against mean values for the same constituents in all ripe samples of all varieties

TABLE 4.—*Ratios between various constituents of ripe fruit, derived from data of table 3*

Variety	Astringent substances to acid	Sugar to acid	Total solids to acid	Total solids to astringent substances	Sugar to total solids	Total solids to tannin	Total solids to nontannin substances	Soluble solids to insoluble solids
Aroma.....	1 : 0.81	1 : 7.8	1 : 16.4	1 : 26.7	1 : 0.47	1 : 45.0	1 : 66.0	1 : 1.96
Parson.....	1 : .51	1 : 6.5	1 : 13.4	1 : 26.2	1 : .48	1 : 45.7	1 : 61.5	1 : 1.97
Sample.....	1 : .59	1 : 7.4	1 : 15.3	1 : 25.9	1 : .48	1 : 48.1	1 : 56.1	1 : 2.01
Portia.....	1 : .47	1 : 7.4	1 : 15.5	1 : 32.4	1 : .47	1 : 65.8	1 : 63.9	1 : 1.91
Progressive.....	1 : .43	1 : 7.4	1 : 15.5	1 : 35.8	1 : .48	1 : 64.0	1 : 81.1	1 : 1.85
Missionary.....	1 : .50	1 : 6.8	1 : 14.1	1 : 28.0	1 : .48	1 : 50.1	1 : 63.3	1 : 2.06
Klondike.....	1 : .39	1 : 5.1	1 : 10.9	1 : 28.1	1 : .47	1 : 44.6	1 : 75.9	1 : 2.26
Dunlap.....	1 : .43	1 : 5.8	1 : 13.7	1 : 31.4	1 : .42	1 : 55.2	1 : 73.2	1 : 1.67
Howard 17.....	1 : .37	1 : 6.4	1 : 12.6	1 : 34.1	1 : .50	1 : 56.2	1 : 88.8	1 : 2.32
Gandy.....	1 : .39	1 : 6.5	1 : 12.3	1 : 31.1	1 : .53	1 : 57.0	1 : 68.8	1 : 2.30
Chesapeake.....	1 : .40	1 : 7.9	1 : 14.6	1 : 36.0	1 : .53	1 : 67.4	1 : 77.5	1 : 2.28
New York.....	1 : .41	1 : 9.9	1 : 17.8	1 : 42.4	1 : .56	1 : 102.3	1 : 72.4	1 : 2.65
All samples.....	1 : .45	1 : 7.0	1 : 14.4	1 : 31.8	1 : .49	1 : 57.0	1 : 72.0	1 : 2.08

The most important of these relationships is that existing between the amount of acid, astringent substances, and sugars present. The balance or the lack of it between these constituents in a fruit is of primary importance in determining its dessert quality. The relative intensity of the effects of these three groups of substances upon the nerves of taste determine the individual's judgment of the fruit as

acid, sweet, tart, or properly balanced. The conception of the basic character of the fruit thus obtained is supplemented by perception through the organs of taste and smell of the mixture of oils and esters which give the characteristic odor and flavor of the species or variety and permit its recognition among others of its class. No matter how agreeable the individual odor and flavor of the variety may be, it will not be considered desirable unless the balance between sugar, acidity, and astringency is such as to be pleasing. In determining palatability, the absolute amounts of these constituents are of minor importance as compared with the ratios existing between them.

The content of total solids in its relation to these constituents is also a matter of some importance in determining dessert quality of the fruit, as a given quantity of sugar, acid, or astringent substance will be more or less apparent as the total solids associated therewith are large or small in amount.

Considered on the basis of the relations existing between total solids, acid, sugar, and astringent content, the varieties included in the study may be combined into four groups: (1) A low-sugar, low-acid, low-astringency group, consisting of Portia and Progressive; (2) a low-sugar, low-acid, high-astringency group, consisting of Aroma, Parsons, and Sample; (3) a medium-sugar, high-acid, medium-astringency group, made up of Gandy, Missionary, Klondike, Dunlap, and Howard 17; and (4) a high-sugar, low-acid, low-astringency group, consisting of New York and Chesapeake. These groups will be discussed in some detail.

LOW-SUGAR, LOW-ACID, LOW-ASTRINGENCY GROUP

Portia and Progressive show a considerable degree of likeness in composition as regards the absolute amounts of their several constituents. Both are low in total solids and in all the constituents of total solids, but notably so in total astringency and its components. As compared with the line of general average (fig. 13), they form a group characterized by low values throughout with especially low astringency. Reference to the table of ratios shows that in respect to the ratios of acid to solids, acid to sugar, and solids to sugar, they do not differ greatly from the second group or from the general average. In the ratio of acid to astringency they are much higher and in that of astringency to total solids much lower than the second group, indicating a materially lower level of astringency in relation to the general level of other constituents than prevails in Aroma, Parsons, and Sample. Their ratios of acid to astringency and to sugar and of astringency to solids are not so low as is the case in New York and Chesapeake, so that their sweetness to taste is not so pronounced as in these varieties.

Progressive is below the general average in every constituent determined except insoluble solids. It is the lowest of the 12 varieties in total astringency, and has lower nontannin astringency than any other variety except Howard 17. In general composition it resembles Portia, but is somewhat higher in total insoluble and soluble solids, sugar, and tannin, and somewhat lower in total astringency and in nontannins. Its low acidity and very low astringency result in a degree of sweetness to taste exceeding that of many berries of considerably higher sugar content.

Portia is lowest of all the varieties in total solids, soluble solids, and acidity. Howard 17 is very slightly lower in insoluble solids, Dunlap is slightly lower in sugar, and Progressive and New York are lower in total astringency. Considered in relation to its other constituents, the insoluble solids and nontannins of Portia are rather high, but its low acid and total astringency ratios give it a sweetness to taste, as in the case of Progressive, out of proportion to its actual sugar content.

LOW-SUGAR, LOW-ACID, HIGH-ASTRINGENCY GROUP

The three varieties Aroma, Parsons, and Sample are very similar. In comparison with the line of reference, or general average (figs. 13 and 14), they are very low in total solids, soluble and insoluble solids, sugar, and acidity, but are quite high in total astringency as a result of a fairly high content of both tannins and nontannins. Reference to the table of ratios brings out the fact that there is also a close resemblance between them in the ratios existing between their constituents. The ratio of acid to astringency is 1 to 0.5 or 0.6; the ratio of astringency to solids approximates 1 to 26, indicating a relatively higher astringency in relation to acidity and to solids than is found in any of the other varieties. The ratio of acid to sugar is slightly above the general average in Parsons, slightly below in Aroma and Sample. The acid-solids ratio is above the general average in Parsons and fairly close to the average in the others. The ratio of solids to sugar is practically identical in all.

Aroma is low in total solids and all components of total solids except astringent substances. It rather closely resembles Parsons in composition, but is slightly higher in absolute amounts of all constituents except acid and nontannin. Sample is considerably below the line of general average in all constituents except astringent substances, in which it is quite high. It is almost identical with Parsons in total solids, soluble and insoluble solids, and sugar, somewhat lower in acidity, and higher in total astringency. It is quite high in nontannin astringency, being exceeded only by Gandy and Missionary in this respect.

Parsons is slightly more acid than Aroma and Sample and slightly less astringent. Like them, it is quite low in total solids, insoluble and soluble solids, and sugar, a fact which determines its place here rather than in the next group. Hedrick (14) characterizes Aroma and Parsons as "pleasantly sprightly" and Sample as "subacid to somewhat sprightly." He terms Portia and Progressive "subacid." These five varieties differ only slightly in their ratios of acid to sugar or to solids, but Portia and Progressive have considerably lower astringency, both absolute and relative, than the others. The term "pleasantly sprightly", as used by Hedrick, evidently connotes the presence in the fruit of such a balance between astringency, acid, and sugar as is pleasing to the palate. With reduction in the amount of astringents present, fruit with the same acid-sugar ratio is subacid, tending toward insipid.

MEDIUM-SUGAR, HIGH-ACID, MEDIUM-ASTRINGENCY GROUP

Gandy, Missionary, Klondike, Dunlap, and Howard 17 (Premier) in that they have high ratios of acid to solids and to sugar, while their ratios of astringency to solids are intermediate between those of

the first and second groups. They are consequently characterized by a sharply acid, distinctly astringent flavor which is subdued but not lost when the fruit is preserved with the addition of sugar.

Missionary is in some respects intermediate in its chemical characters between the last-mentioned group and the present one. It is well above the general average and consequently markedly above the last group in total solids and in practically all constituents of total solids, its total astringency exceeding that of any other variety. In its absolute amount of solids, sugars, and other constituents it is like other members of this group. Examination of the table of ratios shows that in acid-astringency, acid-sugar, acid-solids, and astringency-solids ratios it is almost identical with Parsons. Parsons is a little higher in its acid-sugar and acid-solids ratios than the other members of its group, while Missionary is a little lower in its acid-sugar and acid-solids ratios than the other members of the present group. Parsons and Missionary are consequently intermediate between the low-acid, high-astringency group and the high-acid, medium-astringency group. Missionary is characterized by Hedrick (14) as quite tart, a designation which takes account of its high astringency.

Klondike is considerably above the general average in its total and insoluble solids, is average in sugar, very slightly below average in insoluble solids and nontannins, and markedly high in acidity, total astringency, and tannin. Its high acid-astringency and acid-solids ratios make it relatively the most acid berry of the entire group, despite the fact that its sugar content is up to the general average of all varieties. Hedrick terms it "sprightly to acid."

Dunlap is unique among the varieties studied in that while its total solids, acidity, total astringency, and components of total astringency are all practically on the line of general average, its insoluble solids are the highest found in any variety and its soluble solids and sugars are close to the minimum values found in Portia and Parsons. Its ratio of solids to sugar is the highest noted in the 12 varieties, and its ratio of acid to sugar is exceeded only by that of Klondike. In consequence of its high acid-sugar ratio, it has a greater apparent acidity than other berries of equal absolute acid content but of higher sugar content, such as Chesapeake and Missionary. Hedrick's characterization of the fruit as "mild" is somewhat surprising in view of the chemical data.

Howard 17 (Premier) more closely approximates the general average in the amounts of all its constituents than does any other variety. Total solids, soluble solids, sugar, and tannins fall practically on the general average line; acidity only slightly above it. Insoluble solids and nontannins are the lowest found in any of the varieties, and total astringency is only slightly above the minimum values found in Progressive, Portia, and New York. In the ratios between its constituents, Howard 17 is very much like Gandy, having almost identical acid-astringency, acid-sugar, and acid-solids ratios. All these are higher than average, so that the berry is a distinctly acid one. Hedrick terms Howard 17 "pleasantly sprightly."

Gandy is outstanding in its high content of total solids and of all constituents of the total solids. It is equaled by no other variety in total solids, acid, and nontannins, is exceeded in soluble solids and

sugar only by New York, and in tannin by Klondike, Missionary, and Aroma. As compared with the general average of all samples, Gandy is high in all constituents but especially so in total solids, acidity, and astringency. Its similarity to Howard 17 in the ratios between its constituents has already been noted. Its ratio of solids to sugar, 1 to 0.53, is lower than the average, which has the effect of increasing the sweetness of the fruit to taste. Hedrick classes Gandy as "briskly subacid."

HIGH-SUGAR, LOW-ACID, LOW-ASTRINGENCY GROUP

The New York and Chesapeake present a combination of high solids and sugar content with very low ratios of acid and astringency to solids and especially to sugar. This gives both berries a pronounced sweetness which is agreeable in the fresh condition but which renders them rather characterless and lacking in acidity and sprightliness when preserved with the addition of sugar. Both berries have low ratios of solids to sugar, which further intensifies the sweetness of the fruit.

Chesapeake is high in total and soluble solids and in sugar, being exceeded in the amounts of these constituents only by Gandy and New York. It is very slightly above the general average in insoluble solids and acidity, and somewhat below it in total astringency and tannin. Its ratio of acid to sugar is rather low, but it is preserved from being insipidly sweet by rather high acid-astringency and acid-solids ratios. Hedrick classifies it as "mildly acid."

New York is unique in that it departs more widely from the line of general average in its composition than does any other variety. In total solids it is exceeded only by Gandy, and in soluble solids and sugars it exceeds all others. Its insoluble solids and acid content are below the general average, and its total astringency and tannin content are the lowest found in any of the varieties. Its nontannin content is well above the general average, being exceeded only by Gandy, Missionary, and Sample. Of all the varieties studied it has the lowest ratios of acid to sugar, astringency and tannin to solids, and solids to sugar. This makes it a variety of outstanding sweetness to taste. Hedrick terms it "mildly subacid."

In conclusion, it should be noted that the foregoing results were obtained from fruit grown in one locality only, and that there is a possibility that repetition of the work in a more northern or southern locality would result in an alteration of the relative positions of some of the varieties. These results can be said to hold strictly only for the vicinity of Washington, D. C. There is need for further work upon the effect of various soils and different environmental complexes upon the composition of the strawberry.

CHEMICAL AND PHYSICAL CHARACTERS OF THE STRAWBERRY IN RELATION TO ITS UTILIZATION

The varieties used in this study include all the older and more widely cultivated commercial sorts. They therefore represent the result of selection definitely directed toward the production of fruit of a character adapted to market and shipping purposes, and presumably conform rather closely to the general conception on the part of breeders and growers as to what a desirable commercial berry should be.

These varieties present very great diversity in the degree of their adaptability or suitability for preservation by canning, freezing, or other means, and yield products differing greatly in appearance and palatability when so treated. The reason is that the chemical and physical characters which must be possessed by a berry if it is to yield a satisfactory canned, frozen, or preserved product are in part distinct from those that are acceptable in a commercial or dessert fruit.

Some characters which are unobjectionable or even desirable in a commercial berry are distinctly undesirable in a fruit to be used for preserving purposes. Conversely, some characters which are indispensable to the making of a high-grade preserved product are not required and may even be objectionable in a dessert fruit. The analytical data supply a measure of some of these characters; others show no correlation with any facts brought out by the analyses.

In the following paragraphs an attempt is made to state the characteristics of an ideal berry for preservers' use, to check the different varieties against this ideal, and to correlate chemical and physical composition with adaptability to preserving purposes.

FIRMNESS OF FLESH

In order to be suitable for preservers' use, a strawberry must have a texture of flesh that will enable it to withstand heating in sirup, or freezing and subsequent thawing, without breaking down and losing its form in the process.

Of the varieties herein employed, Klondike and Gandy retain their form under preservative treatments in a higher degree than do the others. The only chemical character of these berries with which this property of resistance to heat appears to be correlated is their high absolute acidity, approximating 1 percent, and their high ratio of acid to other constituents. Whether the high acidity of these varieties is a coincidence or has a causal relation to their behavior in cooking can only be determined from more extensive studies on a wide variety of material. There is clearly no correlation between firmness under these treatments and amount of total solids or insoluble solids or ratios of insoluble to soluble or to total solids.

The puncture tester employed in the present work determined the resistance of the fruit to penetration by a small plunger, 0.032 inch in diameter. At a given stage of ripeness, as red ripeness, all the varieties showed practically equal resistance to puncture, no significant differences being observed among them. Resistance to puncture by a small plunger is definitely correlated with stage of maturity, as shown in a preceding section, but does not show any correlation with the resistance of the fruit to deformation or crushing under pressure or with the amount of softening that will occur when the fruit is heated. The amount of softening and breaking to pieces occurring in the course of preservative treatment cannot be predicted with certainty from the characterizations of varieties in horticultural descriptions as "soft", "moderately firm", and the like, since varieties classed as "soft" and "firm" may break down to the same degree in preserving.

COLOR

The character and degree of change in color occurring in the course of preservative treatment are quite important in determining the

desirability of a berry for preservers' use. An ideal berry for preserving would be one that would retain the color of the fresh fruit unchanged through the preservative processes. No existing berry does this, but both the degree and the character of the change in color differ greatly in different varieties. One of the objects of the present study was to ascertain whether any definite relationship exists between chemical composition and the alteration and loss of color in the process of preservation. The results have been negative, but a brief statement of some of the observed facts may be made.

The pigment of the strawberry shows the general behavior of the anthocyanins, but as its chemical composition has never been determined it is not known whether it is a single pigment or a mixture. Because of its reducing properties it is included in the substances designated as nontannin astringents, along with varying amounts of other and unknown substances which reduce potassium permanganate. There is consequently no relation between the amount of the nontannin astringency and the apparent intensity of color of the fruit. Progressive, Missionary, and Sample are varieties that are deeply colored both at the surface and throughout the flesh to an approximately equal degree. Missionary and Sample have the maximum amounts of nontannins found in the series, whereas Progressive has very slightly more than the minimum found in Howard 17. The other varieties studied differ widely in their apparent pigment content without any relation to their nontannin values.

Darrow and Waldo (9) have studied the color changes occurring in a number of strawberry varieties, including Aroma, Chesapeake, Klondike, Howard 17, Missionary, and Dunlap, and in a number of their own selections. Their colored plate (*pl. 2*) shows that considerable alteration of color occurs when the ripe fruit is picked and allowed to stand for a few days at summer temperature. In the case of Howard 17, Missionary, and Dunlap the change of color is toward purple; in Aroma, Chesapeake, and Klondike it is toward dusky reddish brown. Similar changes occur when the fruit is allowed to become overripe on the plant.

Culpepper and Caldwell (5) have reported that in the course of canning experiments with about 200 varieties and strains of strawberries they encountered several types of behavior of the pigment of the berry after canning. In the type of most frequent occurrence the original red color became converted to some shade of brown or brownish red when the fruit was heated either in glass or in metal cans. This was attributed to isomerization of the pigment to a colorless form together with browning due to the oxidation of tannin. In another type the change in color was a fading, or lessening in intensity, of the red color with no pronounced browning, which would occur if isomerization of pigment without oxidation of tannin had taken place.

In the varieties herein studied there were very considerable differences in the degree and character of the color changes of the canned product. In Chesapeake, Gandy, Howard 17, Missionary, Sample, and Progressive, the outstanding change was a fading, or lessening in intensity, of the original color, apparently due to conversion of some of the pigment to the colorless form. This was accompanied by a variable but never extreme degree of browning. In Aroma, Portia, Parsons, and Klondike the original color was deepened to a dark

brownish red, very similar in appearance in all. In Dunlap and New York there was only slight fading and very little browning.

These results cannot be correlated with any chemical characters brought out by the present study. The group in which pronounced fading under heat occurs includes the deeply colored, highly acid, highly astringent Gandy, together with berries of medium to low acid and astringent content. The group in which pronounced browning occurs contains the highly acid, rather astringent Klondike with others of low acidity and astringency. Dunlap and New York, in which a minimum of change of either type occurs, are quite unlike in chemical characters. It is consequently impossible to correlate behavior of pigment with any type of chemical composition. Understanding of the behavior of pigments of the fruit must await studies of its chemical composition and properties.

PERSISTENCE OF FRESH FRUIT FLAVOR

The palatability of a preserved berry is determined to a very considerable extent by the degree to which the consumer recognizes in it persistence of the flavor of the fresh fruit. This flavor is due partly to essential oils and esters and partly to the balance between sugar, acidity, and astringency in the product. In the process of preservation very considerable quantities of sugar are added to the fruit in the form of sirup. The resulting interchange with the tissue fluids of the fruit brings about considerable change in the flavor of the fruit insofar as the balance between sugar, acidity, and astringency is concerned. As sugar content is increased, acidity and astringency are decreased by dilution with the neutral sirup, and there is also some actual loss of astringent substances through oxidation or destruction by heat in the course of application of the preservative treatment. The extent of these changes will depend upon the amount and concentration of the sirup used and other varying factors, but the direction of the changes will always be toward loss of the balance and character of the fresh fruit and its replacement by a bland, insipid sweetness.

The standards of quality applied to commercial berries are based upon the appeal that the fruit makes when eaten in the fresh condition. In fruit to be so eaten, sweetness is a primary consideration, and a high ratio of sugar to total solids with medium to low proportions of acidity and astringency is desirable. Consequently, the trend in selection of berries for commercial cultivation has tended to eliminate strains that are too high in acidity or astringency, or both, to be agreeable when eaten out of hand. The result is that when a desirable dessert fruit is preserved in sirup, it may become little more than a vehicle for sugar. In order to produce a preserved product which will be strongly suggestive of the fresh fruit flavor it is necessary to balance the added sugar by employing fruit of considerably greater acidity and astringency than would be acceptable in a dessert fruit. Since breeders have heretofore discarded such strains as they have appeared, this necessarily means that in the future attention will have to be given to the development of varieties specifically adapted to preserving purposes through selection of seedlings possessing desirable chemical as well as physical and horticultural characters.

With all the varieties herein studied, some improvement in the balance and flavor of the preserved material was obtained by employing fruit at the red-ripe stage as compared with fully ripe fruit. The obvious reason is that sugar content is increasing and acid and astringent content are decreasing from the stage of red-ripeness onward to full ripeness. These changes result in a decided improvement in dessert quality but entail a very marked decline in character and balanced flavor of the canned or frozen product.

Of the varieties herein studied, the low-sugar, low-acid, low-astringency group, Portia and Progressive, and the high-sugar, low-acid, low-astringency group, New York and Chesapeake, are most seriously lacking in the characters requisite to the making of high-grade preserved products. When preserved by the addition of sugar they become bland, pleasantly flavored, with little or no varietal flavor or character. Whatever other characters they may have which may be considered desirable in a berry for preserving purposes, these varieties serve chiefly as vehicles for conveying sugar.

The low-sugar, low-acid, high-astringency group, Aroma, Parsons, and Sample, yield preserved products of better balance and more character than those just listed. While somewhat deficient in acidity, their relatively high astringency prevents conversion of the product to an insipidly sweet one when preserved in sirup.

The group offering greatest possibilities for preservative purposes, insofar as composition is concerned, is the medium-sugar, high-acid, medium-astringency group, made up of Gandy, Missionary, Dunlap, Howard 17 and Klondike. Of all the varieties herein studied, Klondike is preferred for frozen packing for use in ice cream, etc., and is probably most generally employed for canning, with Gandy ranking next (10). Both combine fairly high acidity and astringency with other desirable characters, including deep-red color extending well into the flesh, and a firmness of texture which prevents cooking to pieces or collapse of the fruit in preserving. Missionary is somewhat similar to Klondike in composition, but is less firm in texture. Dunlap is also somewhat soft as compared with Klondike. Howard 17 is only moderately firm and has fairly good color, but its low astringency makes its preserved products somewhat lacking in sprightliness and balance.

Many or most of the varieties herein studied are rather harshly astringent when under-mature but become less so as they ripen because of the rather rapid reduction in amount of the total astringency. The total astringency is the sum of two components, true tannins and nontannins, the latter consisting of anthocyanins and associated substances having reducing properties. The true tannins considerably exceed the nontannins in the immature fruit but decrease rather rapidly during ripening, the nontannins meanwhile increasing considerably. In the ripe fruit the amount of true tannins is still somewhat in excess of nontannins except in two varieties: Portia, in which the two are practically identical in amount; and New York, in which nontannins exceed true tannins by 50 percent. While both tannin and nontannin contribute to the astringency of the fruit, the true tannins are apparently much more important in determining flavor.

While tannin is a highly desirable constituent, in that it contributes balance and sprightliness to the flavor of the preserved fruit, its presence offers considerable difficulty in canning or freezing. It oxidizes readily upon exposure to the air and more rapidly upon heating, with the production of a brown discoloration. Since the tissues of the fruit contain considerable quantities of air, which is removed with difficulty,⁶ the prevention of brown discoloration in canning and freezing has not been successfully accomplished. It is less rapid and intense in the more acid Klondike and Gandy than in varieties which are lower in acidity, and the experimental addition of an organic acid to the sirup in which fruit of the less acid varieties is canned or frozen results in distinct lessening of the brown discoloration. Consequently, berries that combine high tannin and acid content with a high specific gravity may show less tendency to browning than do berries with low acid and tannin content but with low specific gravity and a high percentage of air in the tissues.

SUMMARY

Studies of the physiological changes in the strawberry fruit occurring during its development were carried on for 4 seasons on 12 varieties, including all the more important commercial sorts of the Eastern States. Samples were collected at intervals of 5 to 7 days, beginning at a time when the petals of the earliest flowers were still attached and continuing until most of the crop had reached full soft ripeness. At the later samplings, material representing all the fruit on the plants was separated into 2 or 3 lots on the basis of size or, in the later stages, of color.

The earliest samples secured were characterized by very marked firmness and by high solids content. As compared with later stages of development, the young fruit at the stage of petal fall has maximum resistance to puncture, maximum total and insoluble solids, and maximum content of material which reduces potassium permanganate (total astringency). Titratable acidity and soluble solids are fairly high but not maximum. Sugars are small in amount and usually make up only 30 to 40 percent of the soluble solids. Considerable sucrose is occasionally present, but free reducing sugars usually make up 60 to 90 percent of the total amount.

On entering upon the phase of rapid enlargement, the fruit decreases considerably in its resistance to puncture. The outstanding chemical change accompanying the onset of rapid growth is a progressive increase in water content. The decrease in total solids is due chiefly to a decrease in insoluble solids, and in smaller part to a decrease in soluble solids. There is a very pronounced decrease in materials that reduce potassium permanganate. Sugar content remains practically stationary or decreases slightly, while active acidity and titratable acidity show an increase.

The period of growth up to the stage of loss of chlorophyll, or beginning of the whitening stage, is one of progressive decrease in resistance to puncture and in total solids, insoluble solids, and astringent

⁶ Culpepper and Moon (?) found that in 36 varieties and strains of strawberries tested the specific gravity of the fruits ranged between 1.008 and 0.922, and the percentages of air by volume in the tissues between 3.3 and 11.3. For Missionary the figures were: Specific gravity 0.965, air 7.2 percent; for Portia, 0.947, 8.3; for Howard 17, 0.946, 8.5; for Progressive, 0.946, 8.8; for Klondike, 0.935; for Dunlap, 0.937; for Aroma, 0.932. They point out that berries of high specific gravity are generally more desirable for canning than those with lower specific gravity, because of the greater tendency toward pinholing of the can in the latter.

materials. Titratable acidity and total sugars usually increase slightly but may fluctuate irregularly with little change. Soluble solids may decrease slightly or show practically no change. In these results the irregular variations in soluble solids and in sugars are in part due to day-to-day variations between rate of absorption of water and rate of formation and transport of sugar into the fruit, and in part to sampling errors.

The stage of whitening is very definitely characterized chemically. At this stage the fruit attains the maximum water content present in its entire life history, together with maximum titratable and active acidity. Soluble solids, which have declined or remained relatively stationary up to this time, now begin to increase, chiefly as a result of increase in total sugars. Insoluble solids, which up to this time have declined fairly rapidly, enter upon a slower rate of decrease. Astringent materials, which have heretofore decreased rapidly, enter upon a much slower and quite irregular decline. Resistance to puncture has decreased to between one-third and one-fourth that found at the initial sampling.

Transition from the stage of whitening to full ripeness is accompanied by a very rapid decrease in resistance to puncture. Total solids increase chiefly as a result of increase in total sugars, which more than compensates for the continuing decrease in insoluble solids. Sugars make up 70 to 80 percent of the soluble solids and about 50 percent of the total solids of the ripe fruit, and consist chiefly of reducing sugars. Titratable acidity declines rapidly; total astringency declines slowly from whitening to full ripeness.

The chemical composition of the crop at any stage in its development or at ripeness is to a very high degree determined by the relative activity of two opposed processes, the absorption of water and the accumulation of sugar. The growth of the fruit is accompanied by progressive hydration, which tends to reduce the percentage content of all the solid constituents. At the same time sugars are being received from the leaves, which tends to increase the solids content. The rate at which hydration occurs is determined by the initial soil moisture and the amount of precipitation; the rate at which transport of sugar to the fruit occurs is governed by temperature and amount of sunlight. Both processes are consequently controlled by environmental conditions. Alternations of conditions that favor one or the other process during growth produce temporary fluctuations in composition of the fruit. Normally the accumulation of water is the dominant process up to the whitening stage; from that time onward accumulation of sugar becomes the dominant process, but the amount of these changes may be altered by external conditions.

These general statements with respect to the chronological sequence of events in the course of development of the fruit are substantially reinforced by a statistical treatment of the analytical data, in which all the 278 samples were considered as one class and in which determinations were made of degrees of correlation between a number of the constituents of the fruit and two different indices of maturity. One of the indices employed, the resistance of the fruit to puncture, or penetration by a needle, is a physical measure of maturity, while the other, the ratio of the insoluble solids of the fruit to the soluble solids, is a chemical measure of maturity. The two measures of maturity have a coefficient of correlation of +0.917, indicating a very

good agreement. Agreement is less perfect with extremely young fruit than with fruit in the later stages of development.

The coefficient of correlation of insoluble solids with resistance to puncture is $+0.882$. The index of correlation of soluble solids with resistance to puncture is 0.641 , and the line of regression is a curve indicating positive correlation when puncture-test values are high, and negative correlation when puncture-test values are low. Total solids plotted against resistance to puncture showed a distinct correlation between the two sets of values, and calculations of the coefficient of correlation gave a value of 0.754 . The index of correlation for total solids with the ratio of insoluble to soluble solids is $+0.884$; the coefficient of correlation of total sugars with resistance to puncture is 0.817 , the correlation being negative; the coefficient of correlation of total sugars with the ratio of insoluble to soluble solids is 0.871 and is also negative. In the case of fruit that has entered the ripening stage, the correlation of both total solids and total sugars with the ratio of insoluble to soluble solids is much closer than their correlation with resistance to puncture. Titratable acidity was found to have an index of correlation of 0.494 with resistance to puncture and of 0.547 with the ratio of insoluble to soluble solids. The forms of the curves indicate little or no correlation for the period prior to onset of ripening, but very good positive correlations with both in the ripening period, that with the latter being somewhat higher. The coefficient of correlation of total astringency with resistance to puncture is 0.926 ; that with the ratio of insoluble to soluble solids is 0.710 , the forms of the curves indicating greater reliability of resistance to puncture as a criterion of maturity in the case of very young fruits. There is a low but significant negative correlation between sugar content and acid content.

As a basis for making comparisons of the chemical composition of the varieties and determining the degree to which varieties have definite chemical individualities, the average composition for all the ripe samples of each variety was determined, and also the grand average for all ripe samples of all varieties. The ratios between certain constituents, namely, acidity and astringency, acidity and sugar content, acidity and total solids, astringency and total solids, and total solids and sugar, were then determined for each of the varieties. Comparisons of these ratios permit grouping of varieties of like composition without regard to the absolute amounts of the constituents present. So considered, the varieties form four groups: (1) A low-sugar, low-acid, low-astringency group, consisting of Portia and Progressive; (2) a low-sugar, low-acid, high-astringency group, made up of Aroma, Parsons, and Sample; (3) a medium-sugar, high-acid, medium-astringency group, consisting of Dunlap, Howard 17, Gandy, Klondike, and Missionary; and (4) a high-sugar, low-acid, low-astringency group, consisting of Chesapeake and New York.

The chemical characteristics of each of these groups as they affect the adaptability of the fruit to various preservative treatments are considered in some detail. None of the varieties studied is especially well adapted to preservers' use, primarily for the reason that the ratios of acidity and astringency to sugar content which are acceptable in a fresh fruit are too low to permit retention of balanced flavor and palatability when the fruit is preserved with sugar. Of the several groups, the medium-sugar, high-acid, medium-astringency group most nearly approaches the type of chemical composition desirable in a berry for preserving purposes.

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THE TREND OF ORGANIC FOOD RESERVES IN ALFALFA ROOTS AS AFFECTED BY CUTTING PRACTICES¹

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INTRODUCTION

For 20 years experimental work relating to the time and frequency of cutting alfalfa (*Medicago sativa* L.) has been conducted at the Kansas Agricultural Experiment Station. The purpose of the earlier experiments was to determine the relation of cutting practices to the yield and the quality of hay. It was thought that frequent cutting stimulated growth and increased the yields. It was soon found, however, that such treatment usually resulted in injury to the stand and decreased yields. Later it was shown that if the alfalfa was not cut too frequently the stands could be maintained longer and the yields increased. Certain data from these experiments also indicated that the latter part of the growing season is the critical period insofar as cutting practices are concerned. It seemed desirable, therefore, to conduct new experiments to verify this conclusion and to study the effect of various cutting practices. Since recent experiments in Kansas and elsewhere have indicated a relation between the time and frequency of cutting and the organic reserves stored in the roots, it seemed advisable to determine the trend of these reserves throughout the season and also the effect of cutting practices on the storage of such reserves. The experiments reported here were undertaken to secure information regarding these points.

REVIEW OF LITERATURE

The literature dealing with the time and frequency of cutting in relation to permanence of stand, yield, and organic reserves has been reviewed by several writers, and an extensive discussion of earlier contributions seems unnecessary. The work of Salmon et al. (17)³ clearly showed that alfalfa is affected by cutting treatments. Graber et al. (6) showed that the vigor and productivity of alfalfa is greatly reduced and the mortality rate of the plants increased by frequent and early cutting. Aldous (2), working with pasture plants, found that the organic reserves of perennial herbaceous plants decrease in the spring until the top development reaches a certain stage, after which they accumulate and are stored in the roots.

In studies with grasses, Graber (5) found that certain practices which interrupted photosynthesis also limited subterranean development, increased the susceptibility to drought and winter injury, and gave other indications of a weakened condition of the plant. Army (3), in his work with the underground parts of five perennial weeds, found

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³ Reference is made by number (italic) to Literature Cited, p. 708.

that the carbohydrate and nitrogenous reserves vary somewhat during the season and that many plants are weakened or killed by certain cutting practices. Previous work by the writer (7) with pasture weeds indicates that if the photosynthetic parts are removed when the reserve supply is low there is a tendency to starve the plant. Kraus and Kraybill (10), Harvey and Murneek (9), Murneek (12), Albert (1), Nelson (13), Pierre and Bertram (15), Reid (16), and others have made distinct contributions to the study of organic reserves.

METHODS AND MATERIAL

The studies reported here are based on two field experiments, the first of which was started in the summer of 1929 in a field of alfalfa that had been seeded the previous fall on a dark alluvial soil of high fertility, and the second in 1931 on a soil of the same type. In the first experiment there were 9 duplicated treatments, differing with respect to time or frequency of cutting, and 6 check plots. The treatments were arranged to give growing periods of various lengths after the last cutting in the fall, thus providing different degrees of winter protection.

Root samples for chemical analyses were taken at the beginning of the experiment, which was the third cutting in 1929, and thereafter at the time the plots were cut until the spring of 1932, except that samples of the first and second cuttings in 1931 were not taken. Root samples were also taken in the late fall and early spring while the plants were dormant. In sampling, all plants were dug in a representative 0.0001-acre area, measured by a hoop. The area was selected from a part of each plot reserved for that purpose, samples being taken from duplicate plots.

The primary objective of the second experiment was to determine more completely than was possible in the first experiment the fluctuations in carbohydrate and nitrogen reserves throughout the season, the relation of these fluctuations to cutting practices, and the effect of the past season's treatments on yields. In 1931, there were 4 variously treated plots, a fifth being added in 1932 and designated as plot 1. Single plots 30 feet wide and 145 feet long were used. In 1932, plot 1 was cut 4 times, each time when in full bloom. In 1931, and 1932 plot 2 was cut first in the bud stage and thereafter 3 times in full bloom; plot 3, twice in the bud stage and twice in full bloom; plot 4, 3 times in the bud stage and once in full bloom; and plot 5, 4 times, each time when in the bud stage. In the fall each plot was divided lengthwise into three divisions designated as *a*, *b*, and *c*. These were treated as follows: On *a* the top growth was removed at the time it ceased to increase in quantity, on *b* the top growth was removed after it had all been killed by freezing, and on *c* the top growth was allowed to remain on the plots for winter protection. It seemed desirable to eliminate the cumulative effect of the treatments; therefore new plots were established each year.

Root samples representative of all plots treated similarly previous to sampling were obtained at weekly intervals. For example, the samples from plot 1 taken from April 2 to May 21, 1932, are representative of plots 2, 3, 4, and 5, as they were all treated alike up to the later date. Likewise, the samples taken on plot 2 between May 21 and June 21, 1932, are representative of plots 3, 4, and 5. Chemical analyses were based on 100 roots selected at random.

The roots in both seasons were analyzed for total carbohydrates and total nitrogen, previous studies having indicated that the effect of the different treatments on root reserves could probably be measured in this way. Immediately after digging, the root samples were cut into 8-inch lengths and washed. Each sample was then cut into pieces approximately 1 inch in length, placed in a glass jar, covered with 95-percent alcohol, sealed, and stored for future chemical analysis. In preparing the roots for analysis the alcohol was drained off and the roots were allowed to dry, after which they were coarsely ground and again placed in the alcohol. They were allowed to absorb the alcohol and were then dried again, the alcohol being evaporated off at approximately 80° C. During the last drying the samples were mixed well with the material in the alcohol by stirring occasionally. The dried samples were again ground until 98 percent or more of the material passed through a $\frac{1}{2}$ -mm sieve. Analyses of total carbohydrates and nitrogen were made of this finely ground material. With this method of drying and incorporating the alcoholic extract with the roots, only one determination for total carbohydrates or nitrogen was necessary for each sample. The Kjeldahl method was used for the determination of total nitrogen. In making the carbohydrate determinations a 3-g sample was hydrolyzed and the reducing power of an aliquot portion was determined in duplicate. The sugar determination was made by the cuprous titration method as developed by Shaffer and Hartmann (18), and the amount of carbohydrates was calculated as dextrose from the Munson-Walker tables (4). Results are reported on a dry-weight basis.

EXPERIMENTAL RESULTS

EXPERIMENT 1

Data secured from the first experiment are given in table 1. It will be noted that at the fall dormant period the differences in total carbohydrates and nitrogen due to the different cutting practices were slight, except in the more severe treatments of plots 14 and 29, which were clipped every 10 days after the last cutting and for which the total carbohydrates and in most cases the nitrogen were consequently less than for any other plot. The difference in carbohydrates ranged from 3 to 10 percent. Plots 12 and 27, which were cut in the bud stage throughout the season, seemed to run somewhat lower at the fall dormant period in carbohydrates than those cut less frequently, but the differences are not great nor are they consistent. Plot series 1, 2, 16, and 17, which was cut but once, and series 4, 5, 19 and 20, and 15 and 30, which were cut but twice during the season, appeared to contain somewhat more carbohydrates and nitrogen than those cut more frequently, but here again the differences are small and not consistent.

The standard error of the experiment was determined from the deviations of the individual determinations from the means presented in table 1 and was calculated by the usual formula $\sqrt{\frac{\sum d^2}{n-1}}$. The standard error for a single determination of total carbohydrates is ± 1.58 percent and of total nitrogen ± 0.08 percent. The figures in the table are averages of 2, 4, and 6 determinations, and this fact must be considered wherever the standard error is applied.

Constituents of roots dug after indicated cutting or at indicated stage—

Plot nos.	1930						1931						1932		Stand 1932						
	Third cutting		Fourth cutting		Fifth cutting		Fall dormant		Spring dormant		Third cutting		Fourth cutting			Fifth cutting		Fall dormant		Spring dormant	
	Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen		Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen	Car- bohy- drates	Nitro- gen
1 and 16)	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	
2 and 17)																					
3 and 18)																					
4 and 19)	39.2	1.41	41.2	1.72			35.7	1.83	28.2	1.95	33.2	1.26	38.2	1.52			35.2	1.61	26.7	2.13	
5 and 20)																					
6 and 21)							36.7	1.88	29.3	2.13							38.9	1.94	27.4	2.50	
7 and 22)	34.6	1.46					35.8	1.73	29.7	1.81	34.5	1.41					37.3	1.72	28.8	2.24	
8 and 23)																					
9 and 24)	37.4	1.73					37.4	1.57	30.6	1.74	32.9	1.51					34.6	1.62	27.3	2.18	
10 and 25)																					
11 and 26)	39.4	1.83					33.7	1.95	27.0	1.83	34.6	1.55					34.4	1.51	28.5	2.23	
12 and 27)	38.2	1.43	37.0	1.25	37.9	1.15	33.7	1.71	29.5	1.79	34.8	1.38	32.9	1.19	33.2	1.47	36.2	1.50	28.4	18.0	
13 and 28)	33.4	1.40					28.1	1.35	25.4	1.67	33.0	1.39					29.1	1.49	25.8	2.06	
14 and 29)																	38.8	1.88	28.0	2.59	
15 and 30)							37.6	1.84	29.2	2.01											

1 Significance of abbreviations: BL, bloom; B, bud stage; FB, full-bloom stage; Seed, seed stage. Leaders indicate that no further cuttings were made.

2 Average of 6 samples taken at random at beginning of experiment, except for plots 12 and 27.

3 Average of 4 samples from plots that had received similar treatment previously.

4 Clipped at 10-day intervals after last cutting.

Data from this experiment relating to stand have been presented in another paper (8), but a summary is given in table 1. It is apparent that, in general, late cuttings, which leave only a short fall growing period before winter, reduced the stands materially below those in which the growing period after the last cutting was longer. The data in table 1 show good agreement between stand and root reserves, except for plots 11 and 26, in which all cuttings were made at full bloom or later, when the root reserves were high.

EXPERIMENT 2

The data from the second experiment, which was conducted in 1931 and 1932, are given in table 2. Very similar results were secured in the two seasons. The data for 1932 are presented graphically in figure 1, the curves having been drawn freehand to represent the

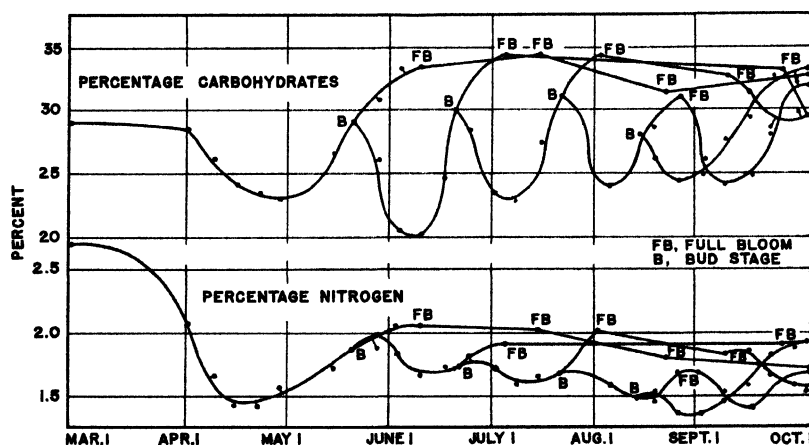


FIGURE 1.—Trend of total carbohydrates and nitrogen during growing season of 1932 as affected by different cutting treatments. Curves were drawn freehand from data in table 2. Dots indicate date of sampling. Dots not intersected by curves are connected by lines to their respective curves.

best fit. It will be noted that when growth starts in the spring and after each cutting there is a rapid decline of total carbohydrates and of nitrogen in the roots. However, the nitrogen curve for a short time after each cutting shows an increase. Probably at that time relatively more carbohydrate than nitrogen is used and hence there is an increase in nitrogen on a percentage basis. This condition is soon changed, and the curve starts down, following closely that of the carbohydrates. Under normal growing conditions, the carbohydrate and nitrogen curves reach a minimum about 20 days after cutting, after which there is a rapid increase, reaching a maximum when the plants are in full bloom. The point of minimum storage appears to depend on the amount of plant growth or probably leaf surface and rate of growth. These relations no doubt depend very materially on temperature and probably on other factors.

DISCUSSION

The effect of the length of the growing period after the last cutting on the accumulation of carbohydrates during the fall dormant period, especially the effect of removing the aftermath at the time growth ceased (Oct. 5), is shown in figure 2. The effect can best be seen by comparing the *b* curves for the different plots and by comparing the *a* and *b* subdivisions for each plot. With respect to the former comparison, it will be noted that the carbohydrate content of the roots

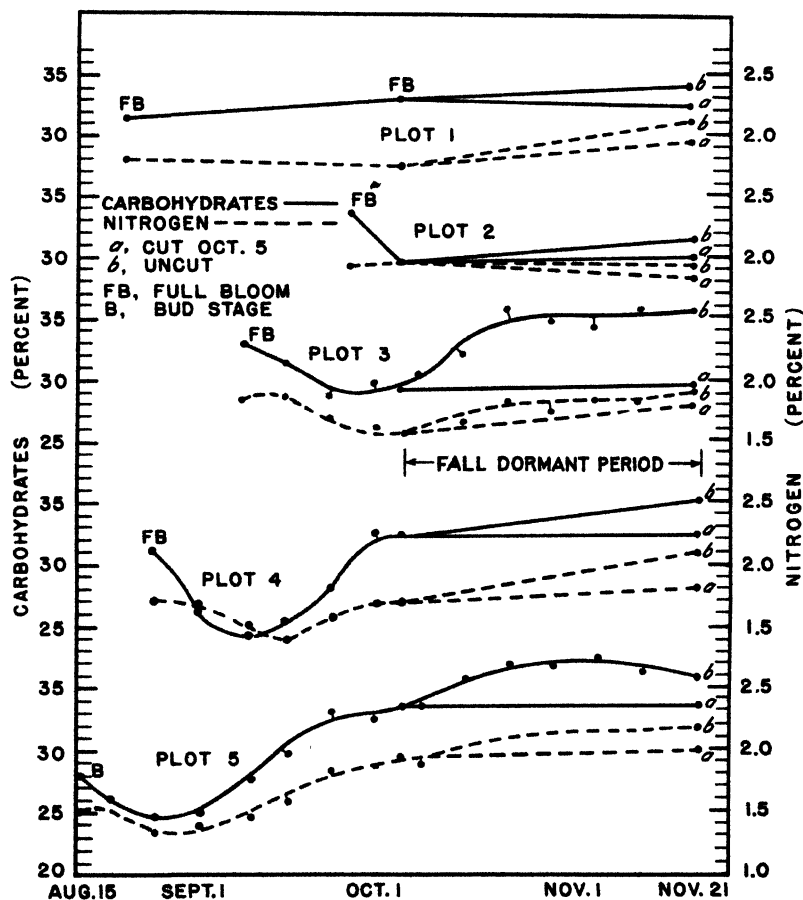


FIGURE 2.—Effect of length of growing period after last cutting in fall and of remaining aftermath on storage of total carbohydrates and nitrogen in roots during fall dormant period, 1932.

at the last sampling date of the season (Nov. 21) was substantially the same for all plots except plot 2, on which it was low. This no doubt is explained by the fact that on this plot the last cutting took place a short period before growth ceased, and the aftermath depleted these reserves but was not sufficient to restore them. On the contrary, the length of the growing period in plots 3, 4, and 5 was sufficient to restore the reserves before November 21. On plot 1

the last cutting coincided with the end of the growing period, hence there was no aftermath and no depletion of reserves.

It will also be noted that in all cases both the carbohydrate and the nitrogen content of the roots on the *b* division, in which the aftermath was not cut until November 21, was moderately greater than on the *a* division of the same plots, on which the aftermath was cut as soon as growth ceased (Oct. 5). These comparisons and the shape of the carbohydrate and nitrogen curves after the last cutting, taken in conjunction with the form of the curves shown in figure 1, seem to afford rather conclusive evidence that the amount of growth which takes place after the last regular cutting has a material bearing on the organic reserves stored in the roots.

It seems that in some cases the last cutting may be too early for a maximum accumulation of reserves. This is indicated by the data from plot 5 in 1931 (table 2), in which the fourth and last cutting was made August 29. On this date the carbohydrate content was 36.4 percent. This increased to a maximum of 39.2 percent on October 28 and afterward decreased to 35.7 percent on December 1. The growing period after the last cutting was long this season and the new growth matured before the beginning of the fall dormant period and a second new growth started from the crowns. No doubt the carbohydrates were decreased as a result. There was no material decrease in 1932 after the date of maximum storage, but in this case the season was shorter and growth ceased about October 5 instead of October 28. The data indicate that the growing period after the last cutting should be only long enough to permit 8 to 10 inches of growth.

Observations of spring growth in 1932 and 1933 indicated some benefit from allowing the aftermath to remain through the winter, as compared with removing it in the early or late fall. Growth started earlier and continued more vigorously throughout the first and part of the second crop. This suggestion is supported by the data in table 3, which shows the effect of these treatments on yield. As compared with *b* plots, in which the aftermath remained until it was killed by frost, the lowest yields were recorded in every case for *a* plots, in which the aftermath was removed as soon as growth ceased; the *b* plots, in turn, in every case but one, produced lower yields than the *c* plots, on which the aftermath remained until spring. Leaving the aftermath on until it was frozen resulted in an average increase of 16.2 percent, and leaving it on until spring resulted in a further increase of 13.2 percent. However, approximately 10 percent of the latter increase was due to the old growth harvested with the new crop, which, when subtracted from that increase (13.2 percent) leaves 3.2 percent due to winter protection. In calculating these averages, plot 1 was omitted because the growth that was left on was equal to a full crop and in farm practice would have been removed as a fourth cutting, as was done on the *a* and *b* divisions; and plot 2 was omitted because there was no aftermath.

The data in figure 1 suggest that the percentage of total carbohydrates at comparable stages of growth during midsummer is lower than in the earlier or the later part of the season when the temperature is lower. This would be expected from the known relations between temperature and the storage of carbohydrates, as pointed out by Miller (11, p. 451), and by Nightingale (14).

TABLE 3.—Effect of previous treatments on yield of alfalfa hay in first cutting

Plot no.	Previous year's treatments					Average of the first cutting, 1932 and 1933		
	Stage of cutting				Fall ¹	Average growth period after last cutting	Height of plants at first cutting	Air-dried hay per acre at first cutting
	1	2	3	4				
						Days	Inches	Pounds
1.....	FB	FB	FB	FB	a	0	14	1,904
	FB	FB	FB	FB	b	0	16	2,287
	FB	FB	FB	(1)	c	47	18	2,887
2.....	B	FB	FB	FB	a	7	14	1,961
	B	FB	FB	FB	b	7	14	2,032
	B	FB	FB	FB	c	7	14	1,815
3.....	B	B	FB	FB	a	22	14	2,017
	B	B	FB	FB	b	22	15	2,317
	B	B	FB	FB	c	22	17	2,385
4.....	B	B	B	FB	a	38	14	2,478
	B	B	B	FB	b	38	16	3,007
	B	B	B	FB	c	38	17	2,220
5.....	B	B	B	B	a	50	15	2,565
	B	B	B	B	b	50	17	2,565
	B	B	B	B	c	50	17	2,943

¹ a, Top growth removed at the beginning of the fall dormant period; b, top growth removed after being killed by freezing; c, top growth left on for winter protection and removed with the first cutting.

² Fourth crop not cut; left for winter protection.

Tables 1 and 2 show a decided decrease in the amount of total carbohydrates at similar stages of development, from year to year. This is probably the result of an increase in the fibrous tissues as the alfalfa roots become older, resulting in a percentage decrease in other constituents. The relation here, so far as nitrogen is concerned, is not so obvious, probably because of the greater effect of stage of growth on nitrogen content.

SUMMARY AND CONCLUSIONS

Twenty years of experimental studies relating to the time and frequency of cutting alfalfa at the Kansas Agricultural Experiment Station have gradually indicated that the fall of the year is the critical period insofar as cutting practices are related to organic root reserves and permanence of stand. Experiments were conducted from 1929 to 1932 in a study of the trend of nitrogen and carbohydrate reserves as affected by various cutting practices during the year, especially in the late fall, and the effect of these reserves on plant survival.

It was found that when growth starts in the spring, and after each cutting, there is a rapid decline of total carbohydrates and of nitrogen until a minimum is reached, after which there is a rapid increase. During the seasons studied, the minimum carbohydrate and nitrogen content was reached about 20 days after cutting. The maximum accumulation apparently occurred when the plants were about in full bloom.

Early and frequent cutting, as in the bud stage, appeared to result in a lower carbohydrate and nitrogen content of the roots when winter arrived, and, conversely, infrequent cutting resulted in a higher content of those reserves. The differences, however, were neither large nor entirely consistent. Clipping every 10 days after the last cutting greatly reduced the nitrogen and carbohydrate reserves and also the stand.

Data were secured which seem to afford rather conclusive evidence that the amount of growth which takes place after the last regular cutting has a material bearing on the organic reserves stored in the roots before winter. If this amount is small the reserves are depleted and there is no opportunity to restore them. It appears that there must be at least 8 to 10 inches of growth to permit maximum storage.

Removing the aftermath when growth ceased in the fall resulted in a lower carbohydrate and nitrogen content than was obtained by leaving this aftermath on the plots.

Permitting the aftermath to remain during the winter appeared to result in a more vigorous growth and an increased yield of the first cutting the following spring.

The percentage of total carbohydrates in alfalfa roots at similar stages of growth decreased as the stand became older.

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YIELD AND CHEMICAL COMPOSITION OF CERTAIN SPECIES OF GRASS¹

By J. G. ARCHIBALD, *assistant research professor of chemistry*, and E. BENNETT, *research assistant in chemistry, Massachusetts Agricultural Experiment Station*²

INTRODUCTION

A search of the literature a few years ago disclosed a scarcity of information on the chemical composition of individual species of grass indigenous to the northeastern part of the United States. Such information would be a valuable adjunct in solving the problem of pasture management, which has been receiving a greatly increased amount of attention in the past 5 years. Accordingly a project was initiated in 1930 for a chemical study of some of the common grasses grown as pure or practically pure stands of the individual species. This paper reports the results of 3 years' work.

EXPERIMENTAL PLOTS AND PROCEDURE

The tract of land chosen for the grass plots is located at the top of a low ridge on the experiment station farm, uniformly level from north to south, but with a slight slope to the west. The soil type is classified as Merrimac fine sandy loam. The surface soil varies in depth from 6 to 12 inches, and is underlain by an orange-brown sandy subsoil grading into loose gravel at from 12 to 24 inches below the ground surface. Mechanical and chemical analyses of the soil are given in table 1.

TABLE 1.—*Results of soil analyses on a basis of air-dry soil*^{1 2}

Profile	Fine soil (1 mm or less)	Organic and volatile matter	Total nitrogen	Total phosphorus	Total potassium	Total calcium	Available-phosphorus	pH
	Percent	Percent	Percent	Percent	Percent	Percent	P p. m	
Surface soil.	92.17	5.06	0.139	0.183	2.01	1.43	154	6.05
Subsoil.	90.33	2.63	.044	.030	1.85	1.44	58	5.50

¹ Samples taken Sept. 20, 1932.

² All determinations except available phosphorus were made according to the following: ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Ed. 3, 593 pp., illus. Washington, D. C. 1930. Available phosphorus was determined by Truog's method.

Previous to 1930 the land had been devoted for years to variety and yield tests on corn or corn and soybeans, and the fertilizer treatment had been uniform for at least 10 years.

¹ Received for publication Oct. 19, 1934; issued June 1935. Contribution no. 205 of the Massachusetts Agricultural Experiment Station.

² Grateful acknowledgment is made of the services of A. F. Spelman and J. W. Kuzmeski, who made the determinations of crude fiber and ether extract under the direction of Phillip H. Smith, chief chemist of the feed inspection laboratory of this station, and also of the services of H. R. De Rose, who made the potassium determinations under the direction of H. D. Haskins, chief chemist of the fertilizer control laboratory of this station.

The species chosen for the investigation were:

Canada bluegrass (*Poa compressa* L.).
Kentucky bluegrass (*Poa pratensis* L.).
Orchard grass (*Dactylis glomerata* L.).
Redtop (*Agrostis alba* L.).
Rhode Island bent (*Agrostis capillaris* L.).
Timothy (*Phleum pratense* L.).
Sheep fescue (*Festuca ovina* L.).
White Dutch clover (*Trifolium repens* L.).

The series was replicated once, making a total of 16 plots. Each plot was 3½ feet wide by 62 feet long, and had an area of approximately one two-hundredth of an acre. Paths 1 foot wide were maintained between the plots with a border 3 feet wide on the margins of the tract. Fertilizer was not applied, as it was desired to ascertain first the composition of the various species grown without fertilizer other than that residual in the soil. The plots were seeded in the late summer of 1930 without a nurse crop, and kept free from weeds by hand work. A reasonable degree of success was attained in keeping the individual species pure. The attempt to secure a pure stand of Canada bluegrass was reluctantly abandoned after three reseeds; competition from other species invariably crowded it out.

Sampling of the plots was done by means of a lawn mower with grass catcher attached. One swath of the mower the entire length of each of the duplicate plots usually provided a sufficient sample; in any case an identical area of the duplicates was always sampled. Occasionally, where patches of other species or of weeds had crept in, the sampling was done by the quadrat method, so that such areas could be avoided. The samples were taken whenever the grass reached a height of 3 to 4 inches, so that time intervals between samplings varied with species and season of the year. Whenever possible a minimum of one sample a month from each species was taken for detailed analysis in each of the 5 months from May through September. Wherever rate of growth necessitated taking more than one sample a month, yield and moisture content only were determined on the additional samples. The entire plot was always mowed immediately after sampling.

Samples were brought to the laboratory and weighed immediately after cutting. After suitable portions had been weighed out for moisture determination, the bulk of the sample was air-dried, then oven-dried at 60° to 70° C., ground in a Wiley mill, and bottled for analysis. The determinations made were moisture, total nitrogen, crude fiber, ether extract, total ash, acid-soluble ash, calcium, phosphorus, magnesium, and potassium. All analyses excepting those for calcium, phosphorus, and magnesium were made according to the official methods of the Association of Official Agricultural Chemists.³ Calcium and magnesium were determined by a modification of McCrudden's⁴ method, and phosphorus by the colorimetric method of Fiske and Subbarow.⁵

³ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. See footnote 2, table 1.

⁴ MCCRUDDEN, F. H. THE QUANTITATIVE SEPARATION OF CALCIUM AND MAGNESIUM IN PRESENCE OF PHOSPHATES AND SMALL AMOUNTS OF IRON DEVISED ESPECIALLY FOR THE ANALYSIS OF FOODS, URINE, AND FECES. Jour. Biol. Chem. 7: 83-100. 1910.

⁵ FISKE, C. H., and SUBBAROW, Y. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. Jour. Biol. Chem. 66: 387-389. 1925.

RAINFALL AND TEMPERATURE

A summary of weather conditions during the growing seasons of 1931, 1932, and 1933 appears in table 2.

TABLE 2.—*Rainfall and temperature at Amherst, Mass., during the growing seasons of 1931-33*

Year	Rainfall						Temperature					
	April	May	June	July	August	September	April	May	June	July	August	September
	In.	In.	In.	In.	In.	In.	° F.	° F.	° F.	° F.	° F.	° F.
1931	2.95	7.44	4.24	3.87	6.57	2.50	46.7	58.1	66.7	73.1	69.6	64.8
1932	2.33	1.67	2.62	3.83	2.67	3.96	44.4	57.9	62.9	68.9	70.5	62.5
1933	5.03	1.69	3.68	2.25	6.63	12.34	45.3	60.5	68.3	72.3	69.3	62.7
Average	3.44	3.60	3.51	3.32	5.29	3.23	45.5	58.8	66.0	71.4	69.8	63.3
Normal (40-year average) ..	3.27	3.63	3.54	4.33	4.24	3.79	45.8	56.8	65.4	70.6	68.4	61.6

¹ Not included in the average because the small number of samples taken in September 1933 were taken early in the month, before these abnormally heavy rains fell.

Except for the month of April the average temperature in all three seasons was consistently above the 40-year normal established for Amherst. Rainfall varied within wide limits, but was consistently below normal in July of all 3 years, which was compensated somewhat by copious rains in August of 1931 and 1933.

PRESENTATION AND DISCUSSION OF RESULTS

Summarized results of the yield and composition of the various grasses appear in table 3.

TABLE 3.—*Summary of results of yields and analyses of the various grass species, seasons of 1931-33*

Species of grass	Samples	Relative yield of dry matter	Moisture in the fresh grass	Analyses on a dry-matter basis								
				Nitrogen	Crude fiber	Ether extract	Total ash	Acid-soluble ash	Calcium	Phosphorus	Magnesium	Potassium
	Number	Pounds	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Kentucky bluegrass ..	10	92	64.59	2.32	25.31	3.37	9.82	5.56	0.49	0.38	0.18	2.13
Orchard grass	14	89	75.73	2.66	23.77	4.67	12.99	9.27	.60	.68	.29	3.51
Redtop	12	104	71.99	2.49	23.32	4.10	10.79	6.99	.62	.35	.21	2.62
Rhode Island bent ..	15	81	67.55	2.50	22.62	3.60	10.36	6.32	.75	.38	.19	2.23
Sheep fescue	15	100	65.26	2.09	28.98	3.19	8.39	4.93	.48	.33	.15	1.92
Timothy	12	91	71.68	2.34	21.23	3.98	8.74	6.16	.50	.36	.15	2.44
White Dutch clover ..	11	140	83.21	4.46	14.82	3.14	11.65	10.15	1.61	.45	.25	2.24

¹ Average of all 7 species=100; expressed in this way because actual yields would be of no significance. In certain months of the several seasons, due to the necessity of reseeding, stands of some of the species were either nonexistent or else so recently established that the growth was insufficient to sample.

Considerable differences are noted in the composition of the several species. Taking the various components in the order in which they appear in table 3, the following points are worthy of mention:

(1) The harsh, wiry texture of sheep fescue is reflected in the lowest moisture content of the group.

(2) There was a close correlation between the nitrogen content of the dry matter and the moisture content of the fresh grass, the ratio for all six grasses being in the neighborhood of 1:30. Unpublished work by the authors and results of other investigators have shown that most of the nitrogen is in the form of true protein.

It will be noted that the nitrogen content of Kentucky bluegrass is somewhat lower, both in actual amount and relative to the other grasses studied, than has been generally reported in the literature. Since this relatively low nitrogen content occurred in 7 of the 10 samples of bluegrass some other explanation than the possibility of analytical error must be sought.

It may be that the soil in question is not a type on which bluegrass thrives well. It has been observed in the course of the work that it was harder to obtain a good stand of bluegrass than of some of the other grasses, and that the bluegrass plots never looked as thrifty as did the majority of the others. It is also possible that the bluegrass did not utilize the residual nitrogen of the unfertilized soil as well as some of the other species were able to; that it is at its best when the level of fertility is relatively high. This suggestion is borne out by data from these same plots for the year 1934. An 8-6-6 fertilizer was applied in the spring of 1934 at the rate of 500 pounds per acre, with the result that the blue grass samples for that year showed a much higher nitrogen content than for the 3 years here reported, the average being 3.51 percent as contrasted with 2.32 percent. This very considerable increase, for the time being, places the bluegrass second on the list in nitrogen content as an apparent result of fertilizer application.

(3) It is of interest to note the correlation between the percentage of crude fiber and the toughness of the grass as determined by breaking strength. Beaumont, Stitt, and Snell⁶ of this station report the following breaking strengths for some common grasses expressed in grams per millimeter of perimeter (average of 50 determinations): Timothy, 58.6; redtop, 52.9; Kentucky bluegrass, 92.6; and red fescue, 147.9.

Except that the timothy had a slightly higher breaking strength than the redtop, these results correlate closely with the amounts of crude fiber reported above. Beaumont concludes that "other factors being favorable, toughness may be a deciding factor in palatability."

(4) The low value for ether extract in white clover is believed to be due to a lower content of wax in this species. Chibnall et al.⁷ have shown that orchard grass has a wax content of 1.20 percent of the dry matter, while the wax content of alfalfa is only 0.35 percent on the same basis. It seems reasonable to assume, therefore, that

⁶ BEAUMONT, A. B., STITT, R. E., and SNELL, R. S., SOME FACTORS AFFECTING THE PALATABILITY OF PASTURE PLANTS, *Jour. Amer. Soc. Agron.* 25: 123-128. 1933.

⁷ POLLARD, A., CHIBNALL, A. C., and PIPER, S. H. THE WAX CONSTITUENTS OF FORAGE GRASSES. I. COCKSFOOT AND PERENNIAL RYEGRASS. *Biochem. Jour.* 25: 2111-2122. 1931.

CHIBNALL, A. C., WILLIAMS, E. F., LATNER, A. L., and PIPER, S. H. THE ISOLATION OF N-THIACONTANOL FROM LUCERNE WAX. *Biochem. Jour.* 27: 1885-1888. 1933.

legumes as a class contain less wax than do grasses, an assumption borne out by superficial observation. Orchard grass, which Chibnall found to contain a relatively large amount of wax, had the highest content of ether extract in the present experiments.

(5) The acid-soluble ash of white clover constituted over 87 percent of the total ash, a proportion much higher than for any of the grasses. Orchard grass was the highest of the grasses in proportion of soluble ash in the total ash (over 71 percent), although timothy was close to it (70.5 percent). Some of the total ash was probably extraneous silica, due to adherent dust on samples taken in dry times or to spattering of soil onto the leaves during heavy rains; but since the clover with its pubescent leaves had an equal opportunity with the rest to become so contaminated, and yet showed such a high percentage of soluble ash in the total, it is believed that the amount of such extraneous material was relatively small in any case.

(6) True to its legume characteristics, the white clover had a very high content of calcium. Of the grasses the two members of the bent family, redtop and Rhode Island bent, were the highest, the latter outstandingly so. The ratio of calcium to phosphorus in these two (1.8:1 and 2.9:1, respectively) is wellnigh ideal from a nutritional standpoint. In this respect the white clover is probably too high (3.6:1), while the orchard grass is low (0.9:1)—another good reason for a mixture of grasses and legumes if optimum grazing conditions are to be approximated. Sheep fescue, Kentucky bluegrass, and timothy were all in the same class as regards both calcium content and the calcium-phosphorus ratio.

(7) The outstanding feature of the values for phosphorus was the high content of it in orchard grass. This high value was consistent through all three seasons, except at the beginning of each season, when the value was just as consistently low. It is associated with relatively high average values for most of the other constituents determined. The form in which this unusual amount of phosphorus exists in the plant and its significance from a nutritional standpoint would be an interesting subject for investigation.

(8) The values for magnesium were quite uniformly distributed through a range from 0.29 percent in orchard grass to 0.15 percent in timothy and sheep fescue.

(9) Orchard grass was much higher in potassium than any of the others, the clover occupying an intermediate position, while the fescue was at the foot again.

SUMMARY

Considering the results as a whole, the outstanding features of the investigation are the high rank of white Dutch clover in almost every respect; the high soluble ash and phosphorus content of orchard grass; the high calcium content of Rhode Island bent; the low rank of sheep fescue in every respect except yield; and the rather low rank of Kentucky bluegrass.

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RATE OF DECOMPOSITION OF ORGANIC MATTER IN NORFOLK SAND AS MEASURED BY THE FORMATION OF CARBON DIOXIDE AND NITRATES¹

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INTRODUCTION

Norfolk sand is extensively found throughout the South and is the predominant type of soil in Florida (2).³ Because of its physical properties and the climatic conditions of Florida, this type of soil is not adapted for retaining organic matter, the addition of which, together with commercial fertilizers, is necessary for the production of maximum crops.

As this study was made in connection with a soil-building program it was thought advisable to study the comparative rate of decomposition of various organic materials in Norfolk sand, under different environmental conditions, with and without the addition of inorganic fertilizers and in the presence and absence of citrus seedlings. Several types of organic materials common to Florida were used, including both legumes and nonlegumes. Leukel, Barnette, and Hester (3) studied the rate of decomposition of various parts of *Crotalaria striata*, one of the plants used in the present work.

EXPERIMENTAL MATERIALS AND METHODS

The materials used in these experiments were stable manure and the plants *Crotalaria* (*Crotalaria striata*), beggarweed (*Desmodium tortuosum*), cowpea (*Vigna sinensis*), and Natal grass (*Tricholaena rosea*). The plants were at the seedling stage; their composition is given in table 1. The entire plant, including roots, was dried in an oven at 65° C. in order that the material might be finely ground and thus insure a more uniform mixture with the soil. These dried organic materials were added to the soil at the rate of 1 percent by weight.

Norfolk sand (deep phase) was used throughout the investigation, and all soil was air dried before the materials were mixed with it and placed in the pots.

¹ Received for publication Dec. 5, 1934; issued July 1935. Part of a thesis presented to the graduate faculty of the Iowa State College of Agriculture and Mechanic Arts in partial fulfillment of the requirements for the degree of doctor of philosophy.

² During the course of this investigation the author has discussed his problems with Drs. P. E. Brown and F. B. Smith of the Iowa State College, and Drs. R. W. Ruprecht and R. M. Barnette of the Florida Agricultural Experiment Station. For their helpful suggestions and criticisms he is grateful.

³ Reference is made by number (italic) to Literature Cited, p. 730.

TABLE 1.—Loss on ignition and composition (percent) of organic matter added to the soil

Material	Loss on ignition	SiO ₂ and sand	N	CaO	MgO	P ₂ O ₅	Na ₂ O	K ₂ O
Cowpeas.....	93.53	1.93	2.44	0.78	0.44	0.85	0.23	0.91
Crotalaria.....	95.51	.65	2.37	.50	.33	.86	.20	1.24
Beggarweed.....	95.33	1.18	1.66	.62	.32	.44	.27	.92
Natal grass.....	89.29	7.61	.71	.16	.27	.74	.20	.80
Manure.....	37.06	54.91	1.01	.33	.33	2.05	.32	1.34

The additions of all inorganic salts were calculated on a basis which would supply plant food equivalent to that contained in 100 pounds of a 4-8-4 commercial fertilizer per acre of 67 trees, or an average of 1.5 pounds per tree. The quantity of inorganic salts added to each pot was in the same relative proportions, per unit area, as the quantity (1.5 pounds) spread around a 1-year-old citrus tree under field conditions. By using 1.5 pounds per tree as a basis, the amounts of inorganic salts required per pot were as follows:

Complete fertilizer { 4.79 g. ammonium sulphate
13.31 g. superphosphate
2.49 g. potassium sulphate

All pots to which ammonium sulphate alone was applied received this material in the same quantity that it was used in the complete fertilizer.

The containers used in the experiment were ordinary 4-gallon, glazed, earthenware pots with a $\frac{3}{4}$ -inch hole in the side near the bottom. This hole facilitated drainage and allowed air to be drawn through the soil when the carbon dioxide in the soil air was to be measured.

To 40 pounds of air-dried soil the materials shown in table 2 were added.

TABLE 2.—Quantities (grams) of material added to 40 pounds of air-dried soil in the various pots

Pot no.	Material added	Quantity added	Pot no.	Material added	Quantity added
1.....	Crotalaria.....	181.44	10.....	Cowpeas.....	181.44
2.....	Beggar-weed.....	181.44		(NH ₄) ₂ SO ₄	4.79
3.....	Natal grass.....	181.44		Manure.....	181.44
4.....	Cowpeas.....	181.44	11.....	(NH ₄) ₂ SO ₄	4.79
5.....	Manure.....	181.44	12.....	(NH ₄) ₂ SO ₄ (check).....	4.79
6.....	Check, no treatment.....	13.	13.....	Crotalaria.....	181.44
	(Crotalaria.....)	181.44	14.....	Beggar-weed.....	181.44
7.....	(NH ₄) ₂ SO ₄	4.79	15.....	Natal grass.....	181.44
	(Beggar-weed.....)	181.44	16.....	Cowpeas.....	181.44
8.....	(NH ₄) ₂ SO ₄	4.79	17.....	Manure.....	181.44
	(Natal grass.....)	181.44	18.....	Complete fertilizer only (check).....	
9.....	(NH ₄) ₂ SO ₄	4.79			

! Plus complete fertilizer.

The materials were weighed out, thoroughly incorporated in the soil, and placed in pots. There was a total of 72 pots, each treatment being made in quadruplicate. Two of these series (36 pots) were kept fallow while the other two (36 pots) were set with two seedlings of *Citrus* (*Citrus aurantium* L.). One fallow pot of each treatment (18 pots) was placed in the greenhouse under somewhat

controlled temperature and moisture conditions, while the other fallow pot of each treatment (18 pots) was placed outside the greenhouse under ordinary atmospheric or field conditions. One pot of each treatment set to citrus seedlings was likewise placed in the greenhouse and one outside. By following this method it was possible to have two series of the soil treatments (as outlined above) in the greenhouse and two outside, one each of these series of 18 pots fallow and one series set with citrus seedlings. After all the seedlings were set, water was added to the pots in sufficient quantity to bring the moisture content up to 50 percent of the water-holding capacity of the virgin soil. This percentage of water (added as tap

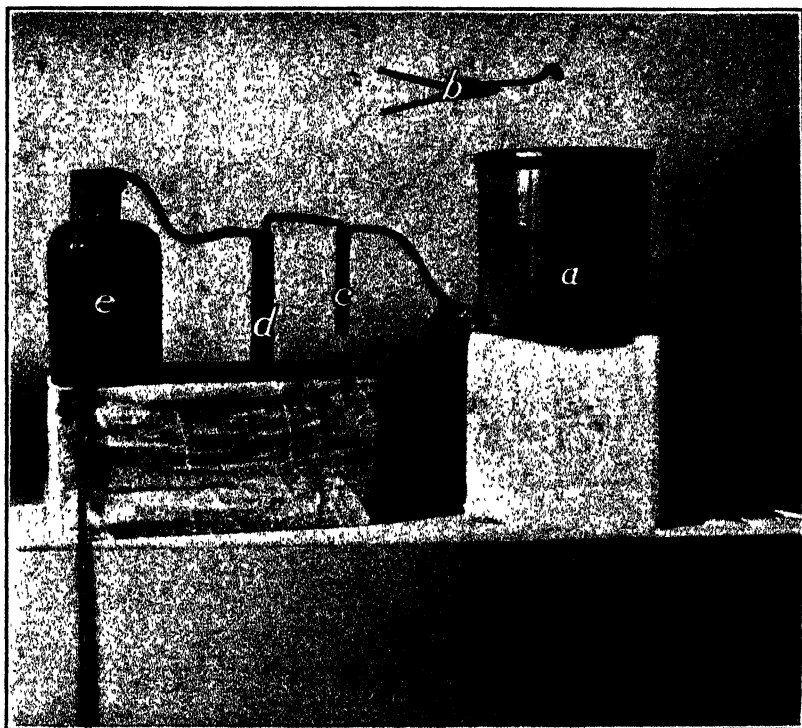


FIGURE 1.—Detail of a single unit of the apparatus used in collecting carbon dioxide: a, Soil pot; b, tube used for collecting the carbon dioxide and drainage; c, test tube containing N/10 solution of hydrochloric acid; d, 100-cc absorption cylinder containing 2N sodium hydroxide; e, 5-l aspirator bottle filled with water.

water) was maintained throughout the experiment in all pots kept in the greenhouse.

The method used for collecting the carbon dioxide was very similar to that employed by Turpin (6). Before the pots were filled with soil a 2-hole rubber stopper was fitted into the hole on the side of the pot near the bottom. Two copper tubes were inserted through the stopper, one to be used for collecting drainage water and the other for drawing air through the soil to displace the soil air. The tube for collecting the carbon dioxide was bent in gooseneck shape with the short bend turned down (fig. 1, b) and was long enough to

reach to the center of the pot. Over these tubes fine rocks were placed in order to give good drainage channels and prevent the tubes from becoming clogged with soil. The soil was placed on these rocks. For collecting the carbon dioxide a special apparatus was used which would allow 5 l of the air to be drawn through the soil in each pot. Figure 1 shows a single unit of the set-up used in this work. Two glass receptacles were placed between the bottle and the pot. These receptacles consisted of an 8-inch test tube and a 100-cc cylinder. A 2N sodium hydroxide solution was placed in the cylinder to absorb the carbon dioxide. The test tube containing N/10 solution of hydrochloric acid was placed nearest the pot. To collect the carbon dioxide a 5-l aspirator bottle was filled with water, the hook-up completed, and the overflow regulated so as to allow the aspirator bottle to be emptied in 1 hour; then the flasks were taken to the laboratory for the analyses. A battery of 10 of these units was run simultaneously. The carbon dioxide was collected at



FIGURE 2. -Set-up of experiment outside the greenhouse.

definite intervals and the quantity determined by the use of the double titration method (1).

All pots outside the greenhouse were buried in the soil to within 1 inch of the top and so arranged as to allow the copper tubes to project through a wall into a pit. The short tubes were bent down and connected with bottles for collecting the drainage water. The tubes for collecting carbon dioxide were connected with the collecting units in the greenhouse by the use of long glass tubes. When the soil was being aerated, the drainage tubes were closed, thereby forcing the air through the soil and displacing the soil air. Figures 2 and 3 show the arrangement of these pots outside and inside the greenhouse.

In determining the carbon dioxide content of the soils 22 separate collections were made. The results thus obtained on different dates were averaged by means of a moving average. In the use of the moving average the quantities of carbon dioxide for three consecutive determinations were added and divided by three. The average thus obtained was considered as representing the carbon dioxide for the middle date. This process was repeated until five periods were obtained for each treatment.



FIGURE 3.—Set-up of experiment in the greenhouse.

EXPERIMENTAL RESULTS

EFFECT OF SOIL TREATMENTS UPON EVOLUTION OF CARBON DIOXIDE IN NORFOLK SAND

The data obtained by the foregoing process are presented in figures 4 to 7. A study of these data shows that the amount of CO_2 formed in the soils to which organic matter was applied exceeded that produced in soils to which no organic matter was applied. The CO_2 evolved from soils treated with legumes exceeded, for the first 27 days, that evolved from soils treated with Natal grass or manure, which is in keeping with the results reported by Waksman and Tenney (?) relative to the influence of the nitrogen content of organic matter. The maximum quantity of CO_2 in every instance was evolved at the time the first measurement was made and thereafter diminished until, at the end of 4 months, there was an apparent constant rate of decomposition, and the amount was equal to that formed from the virgin check soil. The rate of decomposition found here is in accord with the results of Martin (4) and others.

The evolution of CO_2 in the soils kept in the greenhouse was at a maximum during the first 60 days, whereas in the soils kept under field conditions the maximum amount was formed during the first 24 days. The time required for CO_2 formation to become constant outside the greenhouse was about half that required in the greenhouse. This indicates a quicker action on the part of the organisms outside the greenhouse, although the total action was not so great as under greenhouse conditions. From this it is concluded that the greenhouse

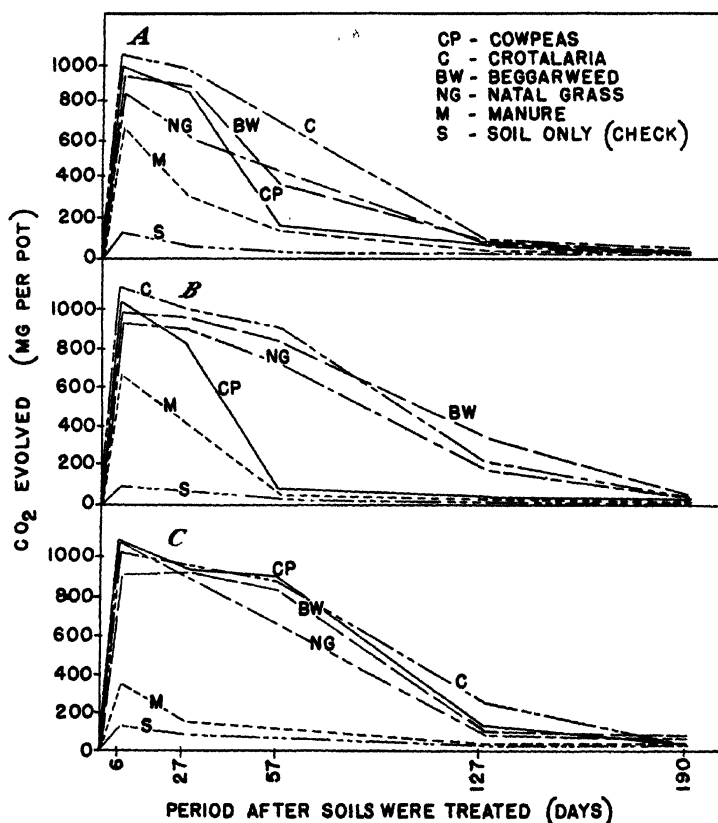


FIGURE 4.—Effect of various soil treatments on the evolution of CO_2 in Norfolk sand, in the greenhouse with seedlings: A, indicated materials added alone; B, indicated materials and $(\text{NH}_4)_2\text{SO}_4$ added; C, indicated materials and a complete fertilizer added.

conditions were more favorable for complete decomposition than were the field conditions.

Figure 8 represents the total CO_2 collected from the soils under each treatment during the entire period of the experiment. These curves show that generally more CO_2 was evolved from the soils bearing citrus seedlings than from those left fallow and that more was evolved from the soils kept in the greenhouse, where optimum soil moisture was maintained, than from the soils outside the greenhouse. The addition of inorganic fertilizer caused an increased

total CO_2 evolution over that of the organic materials alone from practically all the soils containing seedlings. The complete fertilizer and ammonium sulphate alone appeared to be of approximately equal value in this respect. Whether this increase was due to greater biological activity or to increased root growth, it is difficult to say. As there was a tendency toward a reduction in the evolution of CO_2 from the fallow soils to which ammonium sulphate, in the greenhouse,

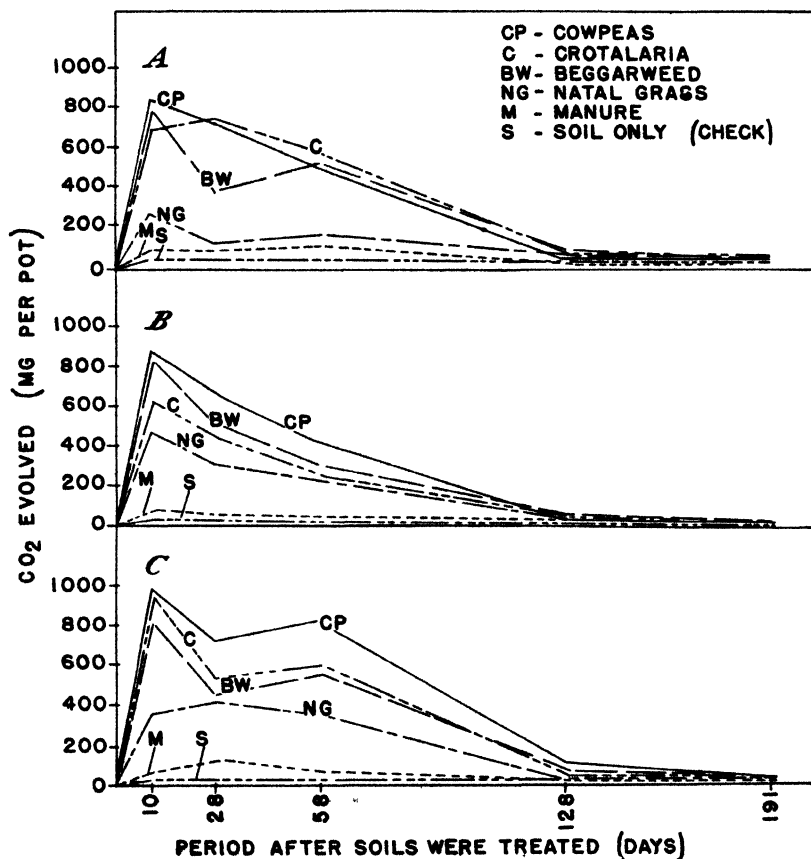


FIGURE 5.—Effect of various soil treatments on the evolution of CO_2 in Norfolk sand, in the greenhouse, without seedlings: A, Indicated materials added alone; B, indicated materials and $(\text{NH}_4)_2\text{SO}_4$ added; C, indicated materials and a complete fertilizer added.

and complete fertilizer outside the greenhouse, had been added, it might be concluded that the increase of CO_2 from the cultivated soils was due to increased root growth. The inorganic fertilizers must have been responsible for increased root growth, the increased root growth possibly having a corresponding effect on the amount of CO_2 evolved. The addition of inorganic salts had no influence in the soils to which no organic matter had been added.

DETERMINATION OF FREE AMMONIA IN SOIL AIR

It was thought advisable to test for ammonia formation in connection with this work. Therefore, 10 cc of a N/10 solution of hydrochloric acid was placed in the test tube between the earthenware pot and the CO₂ absorption cylinder. This process was followed for a

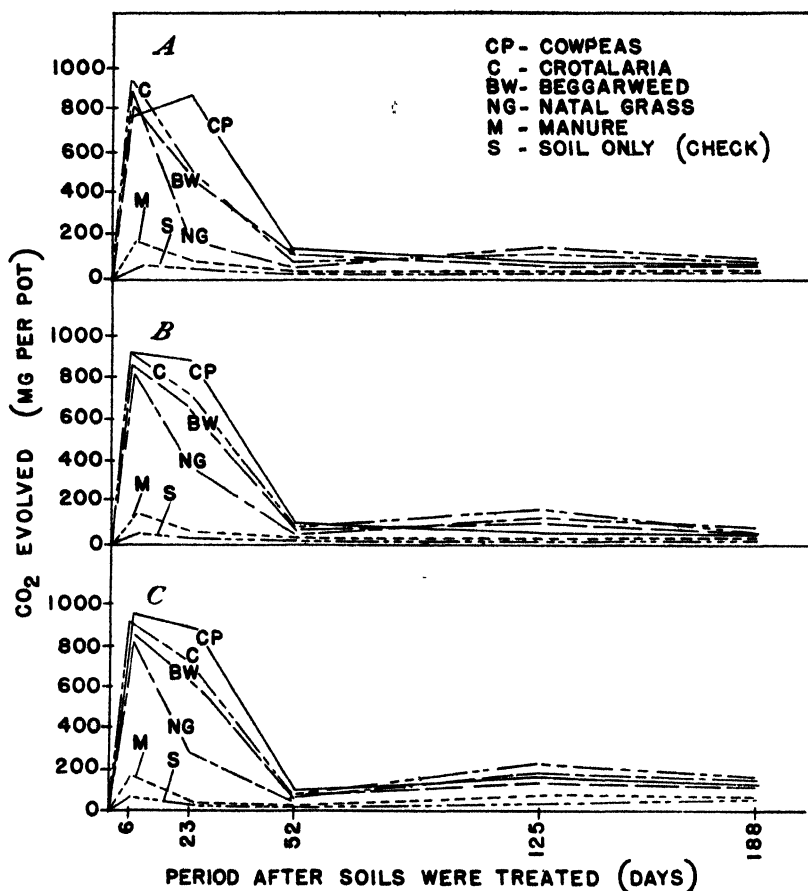


FIGURE 6.—Effect of various soil treatments on the evolution of CO₂ in Norfolk sand, outside the greenhouse, with seedlings: A, Indicated materials added alone; B, indicated materials and (NH₄)₂SO₄ added; C, indicated materials and a complete fertilizer added.

period of about 6 months each time the CO₂ was collected. The time covered in this process considerably exceeded that of the greatest activity of the micro-organisms, as measured by the CO₂ evolved.

After the soil air had been passed through the acid solution, the acid was titrated against N/10 sodium hydroxide solution. At no time during this period was there any indication of loss of nitrogen through volatilization of ammonia.

THE EFFECT OF SOIL TREATMENTS UPON THE ACCUMULATION OF NITRATES IN NORFOLK SAND

The best material for improving the physical or chemical properties (or both) of a soil is not necessarily the one that forms the greatest amount of nitrates or other plant food. It is the one that produces nitrates at a rate only sufficient to feed the crop adequately. Any production in excess of this amount is subject to loss through leaching. Whether or not any nitrogen is made available will depend

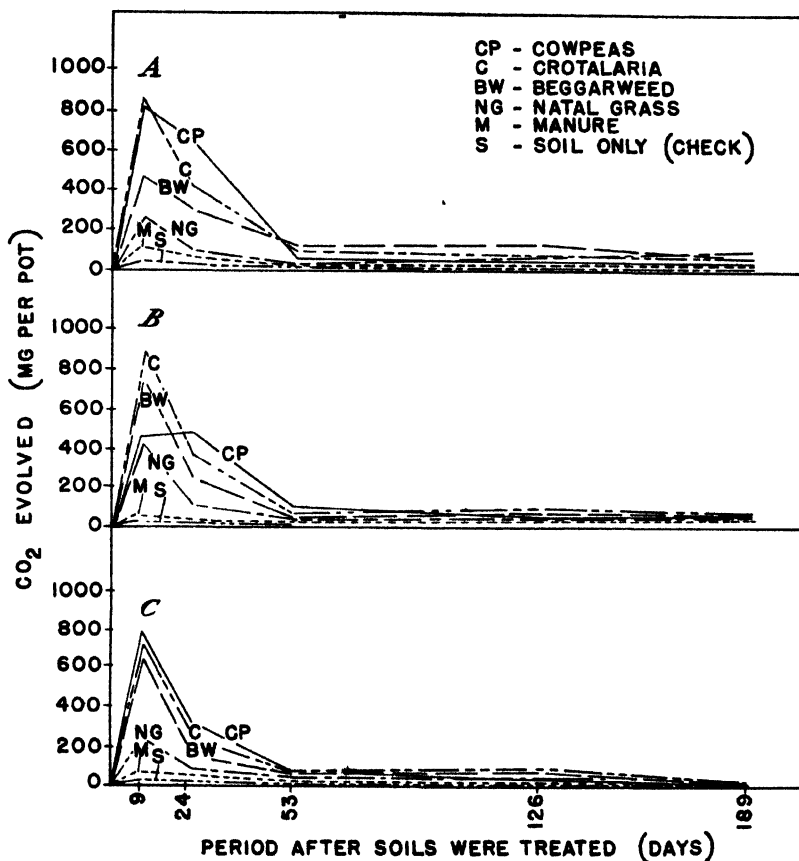


FIGURE 7.—Effect of various soil treatments on the evolution of CO₂ in Norfolk sand, outside the greenhouse, without seedlings: A, Indicated materials added alone; B, indicated materials and (NH₄)₂SO₄ added; C, indicated materials and a complete fertilizer added.

in part at least on the amount of nitrogen in the plant material. Likewise the rate of decomposition will depend on the supply of nitrogen available for the organisms.

As the growing citrus seedlings would assimilate some of the nitrates, the results from the fallow pots only are presented. There being no drainage from the pots remaining in the greenhouse because of the control of the moisture content, only the drainage water from the pots kept outside the greenhouse had to be considered. The

nitrate in the drainage water from these pots was determined each time the water was collected. The quantity of nitrate in the water from a given pot was added to the total quantity of nitrate found in the soil from that pot the next time it was sampled subsequent to the date of drainage. By following this method, all the nitrate could be accounted for and comparisons could be made directly with the quantity found in similar pots in the greenhouse.

At definite intervals the soil in each pot was sampled by the use of a suitable borer and taken to the laboratory for analysis. Twenty grams of this soil were weighed into Erlenmeyer flasks, 100 cc of distilled water added, and the mixture was thoroughly shaken, and after standing for a short time, filtered. The procedure from this point was that of Schreiner and Failyer (5). As in the case of the carbon dioxide studies, 22 nitrate determinations were made on each

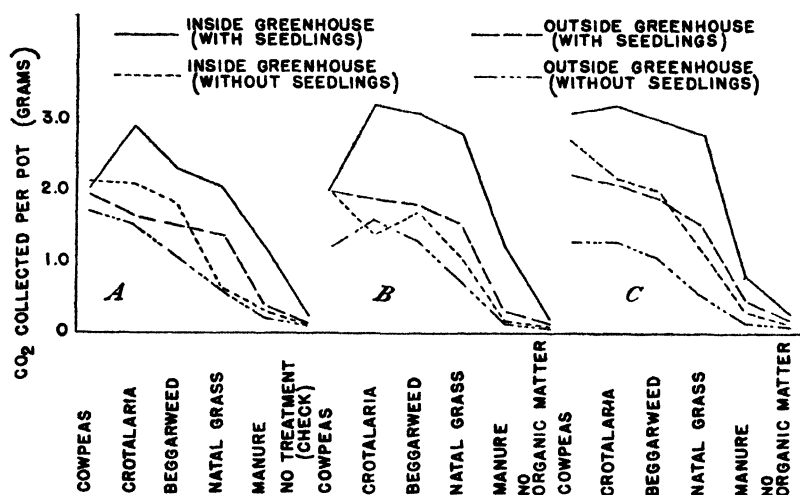


FIGURE 8.—Effect of various soil treatments upon the total CO_2 collected from the soil during the experimental period of 268 days: A, Indicated materials added alone; B, indicated materials and $(\text{NH}_4)_2\text{SO}_4$ added; C, indicated materials and a complete fertilizer added.

soil. The method of averaging the data previously employed was followed in these studies.

It appears that the percentage of total nitrogen in the material which is changed into nitrates might be a better criterion of the value of the material than total nitrate accumulation. The curves presented in figures 9 and 10 have been drawn from data obtained as follows: The amounts of nitrate found at definite intervals, after deducting that found in the respective checks, were divided by the actual weights of nitrogen (calculated as nitrates) added to the soil in the organic matter, or inorganic fertilizer. The curves show the actual percentage of nitrate found in the soil at certain intervals, which was derived only from the organic materials.

The zero (0) line in the figures represents the quantity of nitrate found in the virgin soil. The curves above the zero line represent the percentage of nitrate accumulated from the organic-matter treatments alone, and the curves below the zero line represent nitrate

assimilation. The curves for ammonium sulphate and complete fertilizer show the quantity of nitrate produced from these materials only. The curves bring out the difference in the ability of the various types of organic and inorganic materials to produce nitrates under various conditions.

The maximum nitrate accumulation in the greenhouse and in pots kept under field conditions occurred at different times, and the actual percentage of nitrogen changed to nitrates was different. Not only

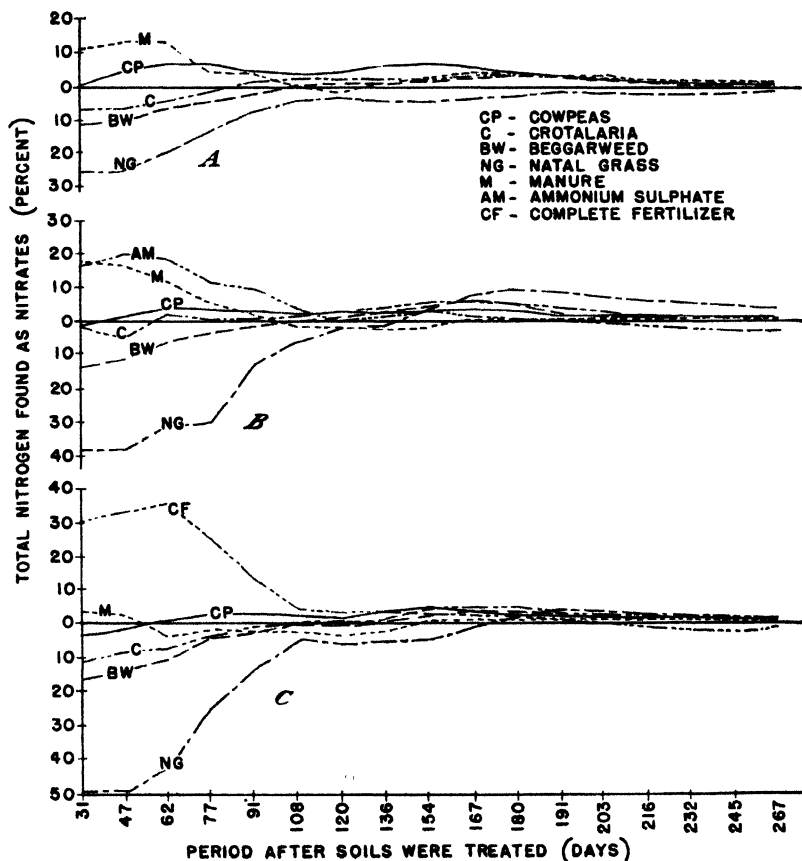


FIGURE 9.—Effect of various soil treatments on the formation of nitrates in the soil, outside the greenhouse, without seedlings: A, Indicated materials added alone; B, indicated materials and $(\text{NH}_4)_2\text{SO}_4$ added; C, indicated materials and a complete fertilizer added.

was the period of greatest nitrate accumulation more prolonged in the soils in the greenhouse, but the percentage of nitrogen transformed was also greater. The maximum transformation from all the organic materials in the soils kept outside the greenhouse occurred after the end of 3 months, while for the same treatments in the greenhouse maximum transformation occurred much earlier.

When the individual materials which were kept under field conditions are considered (figs. 9, 10) it is found that the rate at which nitrates were formed from nitrogen in the various materials, in the

order of decreasing percentage, was as follows: Complete fertilizer, ammonium sulphate, manure, cowpeas, crotalaria, beggarweed, and Natal grass, regardless of any additional treatment. This is what

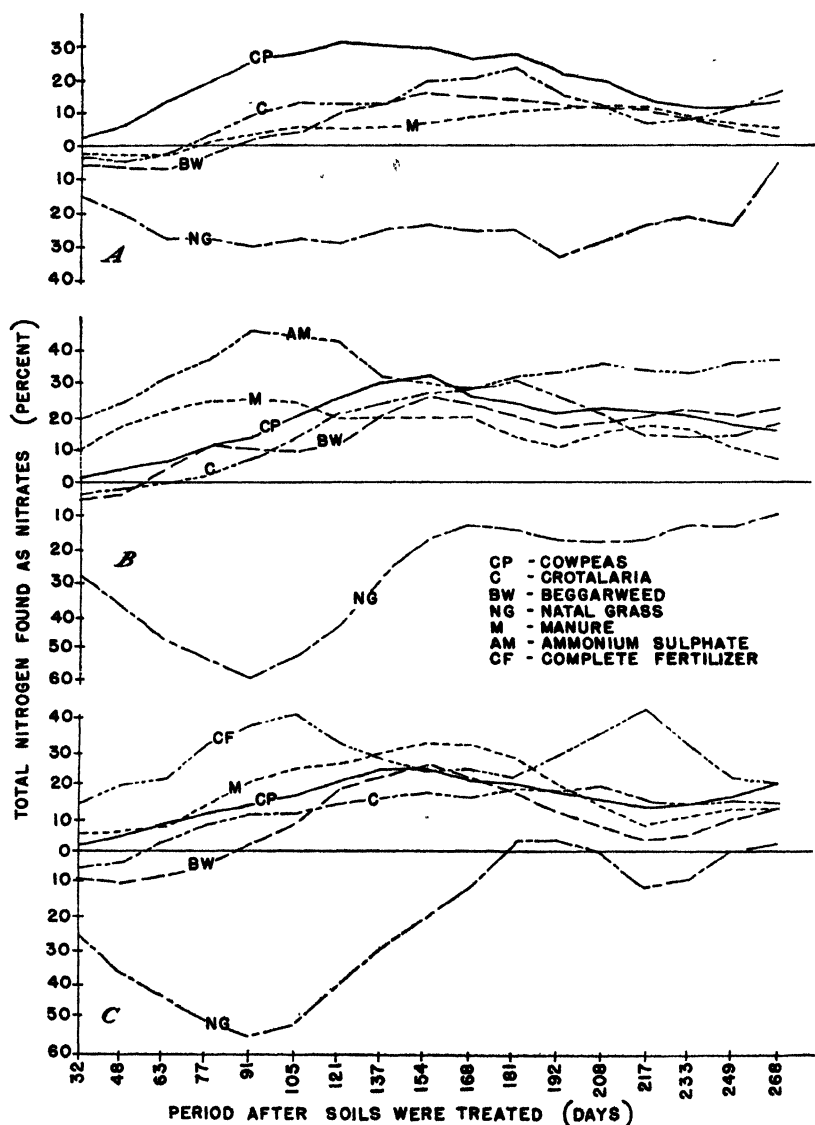


FIGURE 10.—Effect of various soil treatments on the formation of nitrates in the soil, in the greenhouse, without seedlings: A, Indicated materials added alone; B, Indicated materials and $(\text{NH}_4)_2\text{SO}_4$ added; C, Indicated materials and a complete fertilizer added.

would be expected from the nature of the materials. The only significant difference in the rate of nitrate accumulation from the organic materials, as brought about by the addition of inorganic salts, was in the case of Natal grass. This, too, would be expected since the

organisms would naturally utilize the nitrogen in inorganic fertilizers first, as it occurred there in a more available form. This effect on Natal grass was also observed under greenhouse conditions.

The curves representing the data secured under greenhouse conditions (fig. 10) indicate that the nitrogen in the complete fertilizer and that in ammonium sulphate were changed to nitrates at practically the same rate and that the nitrogen in these materials was converted more rapidly than that in any of the organic materials. In rapidity of oxidation of nitrogen the organic materials ranked as follows: Cowpeas, manure or cowpeas treated with the inorganic salts, cro-talaria, beggarweed, manure alone, and Natal grass.

Apparently some combination of Natal grass and a nitrogenous inorganic material, such as ammonium sulphate, should be a satisfactory combination of materials to add to the soil, for this combination showed the smallest percentage oxidation of the nitrogen from the organic material.

SUMMARY AND CONCLUSIONS

A study was made of the comparative rate of decomposition of various organic materials in Norfolk sand and of the effect of the addition of inorganic fertilizers on the rate of decomposition of these materials.

The amount of CO_2 evolved and the nitrates accumulated were used to measure the rate of decomposition of organic matter. The CO_2 evolved from the soils treated with legumes exceeded for the first 27 days the CO_2 evolved from the soil treated with Natal grass or manure. In every instance the maximum quantity of CO_2 was evolved at the beginning of the experiment and gradually diminished thereafter. There was an apparent constant rate of decomposition, as measured by CO_2 evolution, at the end of about 4 months, when the amount formed in the treated soil was approximately equal to that formed in the virgin soil. The period of maximum CO_2 evolution in the case of the soils kept in the greenhouse occurred during the first 2 months of the experiment, whereas that for soils kept under field conditions was formed during the first 30 days.

More CO_2 was evolved from the soils bearing citrus seedlings than from those kept fallow, and the greatest quantity of CO_2 was produced from the soil kept in the greenhouse. The addition of inorganic nitrogenous fertilizers increased the amount of CO_2 given off from all soils bearing citrus seedlings but this increase was possibly due to the increased root growth.

There was no evidence of any loss of nitrogen from these soils through volatilization of ammonia.

In the soils treated with Natal grass there appeared to be considerable nitrogen assimilation. A striking difference in the availability of the nitrogen in the organic materials under different conditions was noted. A greater percentage of the nitrogen in the organic materials was made available under greenhouse conditions, and this greater availability was distributed throughout the period of the experiment.

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THE EFFECT OF GRINDING ON THE DIGESTIBILITY OF CORN BY PIGS AND ON ITS CONTENT OF METABOLIZABLE ENERGY¹

By W. P. GARRIGUS, *graduate student*, and H. H. MITCHELL, *chief in animal nutrition, Department of Animal Husbandry, Illinois Agricultural Experiment Station*

INTRODUCTION

The effect of grinding on the value of corn (*Zea mays*) as a feed for pigs has been studied mainly in feeding experiments, the results being measured by the rapidity and feed economy of the gains secured. In the United States it has in general been found, according to Morrison,² that for young pigs ground corn is not appreciably superior to whole corn, but for older pigs, variable percentage advantages in economy of gains may result from the feeding of ground corn. However, a more recent summary by Crampton³ indicates no advantage due to grinding at any weight of pig. In sharp contrast to the American experiments are the results of Hansson⁴ in Sweden, who calculated from his own feeding experiments on pigs that the productive value of maize was increased by fine grinding by as much as 15 to 18 percent.

Such findings as are obtained in feeding experiments are the resultants of a number of factors, including effects of grinding on the digestibility of the corn, on its palatability, and, as Crampton points out, on its wastage at the feed trough: "Shelled corn lost from the feeder is salvaged by the hogs but ground feed is not * * * Feed rooted out of the trough and lost to the pig is, of course, still charged to the gains made." A complete understanding of the problem, therefore, demands a consideration of each of these factors.

PREVIOUS INVESTIGATIONS

The factor most amenable to quantitative study, and perhaps the most fundamental factor of all, is the effect of grinding corn on its digestibility. However, very little study seems to have been made on this point. A summary of such work as has been found in the literature is embodied in table 1.

The corn in Jordan's experiment⁵ was a flint variety. The experimental pig weighed about 50 pounds and received in each of the two digestion trials 787 g of corn daily. The collection period was of 5 days duration.

¹ Received for publication Feb. 18, 1935; issued July 1935.

² HENRY, W. A., and MORRISON, F. B. FEEDS AND FEEDING; A HANDBOOK FOR STUDENT AND STOCKMAN. Ed. 18, 770 pp., illus. Madison, Wis. 1923.

³ CRAMPTON, E. W. THE COMPARATIVE FEEDING VALUES FOR LIVESTOCK OF BARLEY, OATS, WHEAT, RYE, AND CORN, A REVIEW AND ANALYSIS OF PUBLISHED DATA. * * * 107 pp., illus. Ottawa. 1933. (Canada Honorary Advisory Council Sci. and Indus. Research Rept. 28.)

⁴ HANSSON, N. EINFLUSS DER VERSCHIEDENEN BEKEITUNGSWEISEN AUF DER VERDAULICHKEIT UND DEN FUTTERWERT DER GETREIDEARTEN. Biedermann's Zentbl. Agr. u. Rationellen Landwirtschaftsbetrieb, Abt. B. Tierernährung, Ztschr. Gesamt. Fütterungslehre u. Futtermittelkunde 3: 243-274. 1931.

⁵ JORDAN, W. H. DIGESTIBILITY OF MAIZE KERNEL (CORN) IN VARIOUS FORMS. Maine Agr. Expt. Sta. Ann. Rept. 1885-86: 59-64. 1886.

In Haberhauffe's experiment,⁶ the degrees of fineness of the ground corn studied can be roughly characterized by the percentages passing through a 1-mm round-hole sieve, i. e., 42, 63, and 98. The two pigs weighed initially 87.5 kg. They received daily 4 kg of the corn mixed with water, except that the whole corn was fed mixed with the coarsely ground corn in even proportions. The digestibility of the whole corn was then computed indirectly. Apparently the pigs were not fed or confined separately during the collection period of 7 days.

TABLE 1.—*Previous investigations of the effects of grinding on the digestibility of corn when consumed by swine*

Condition of corn	Number of pigs	Digestion of coefficients obtained					Authority and reference
		Organic matter	Crude protein	Crude fat	Crude fiber	N-free extract	
Whole kernels	1	83.4	68.7	45.6	38.3	88.8	Jordan ¹
Finely ground	1	91.2	86.1	81.7	29.4	94.2	
Whole kernels	2	89.6	78.6	51.7	52.4	94.7	Haberhauffe, ²
Coarsely ground	2	90.4	79.2	59.7	55.7	95.0	
Medium ground	2	93.7	82.4	79.0	75.9	96.8	
Finely ground	2	94.9	87.2	78.7	78.0	97.5	

¹ See footnote 5.

² See footnote 6.

The experiment of Jordan⁷ in particular indicates a marked improvement in the digestibility of corn by grinding it to a fine meal, possibly because a flint variety of corn was used. In Jordan's study, grinding improved the digestibility of the organic matter 9.4 percent, that of protein 25.3 percent, and that of nitrogen-free extract 6.1 percent. The results obtained by Haberhauffe indicate less improvement for the finely ground meal, i. e., 5.9 percent in the digestibility of the organic matter, 10.9 percent in the digestibility of the protein, and 3.0 percent in the digestibility of the nitrogen-free extract. The meals of intermediate fineness show an intermediate but graded improvement.

It appears from these two experiments, constituting the only published work on the problem, that the effect of grinding on the digestibility of corn has not yet been precisely measured. Since the nutritive advantages of the grinding of corn must be balanced against the cost of grinding in deciding whether and when this method of preparing corn is economical, the need of more work of this character seems evident. The experiment reported below is a further contribution to this problem.

PLAN OF EXPERIMENT

The subjects of the experiment were 5 Chester White barrows and 1 Hampshire barrow (pig 4), weighing initially from 135 to 196 pounds. Each of the pigs received daily either 1,300 g or 1,500 g of corn, depending upon appetite, with no other feed. The digestibility and metabolizable energy content of the whole corn and of the same corn after grinding was determined with 5 of the 6 pigs, 1

⁶ HABERHAUFFE, W. ÜBER DEN EINFLUSS DER ZUBEREITUNG AUF DIE VERDAULICHKEIT DER FUTTERMITTEL. Jour. Landw. 74: [191]–230. 1926.

⁷ JORDAN, W. H. See footnote 5.

pig refusing to consume the whole corn in adequate amounts after a feeding period on ground corn. Three of the pigs received the whole corn first, and three received the ground corn first. In an immediately following period, the rations were reversed, each pig continuing, however, on the same amount of feed.

Each feeding period consisted of from 14 to 17 days, during the last 10 days of which both feces and urine were collected. The animals were confined throughout in metabolism crates very similar in size and design to the crate described by Forbes.⁸ The feces of the collection periods were separated from those of adjacent periods by giving ferric oxide (equal in weight to 3 percent of the morning feed) to the pigs on the morning of the first day of the collection and again on the morning of the day following the termination of the period. The feces were dried at a low temperature and analyzed for moisture, nitrogen, crude fiber, and gross energy (heat of combustion), while the urines (with washings) were analyzed for nitrogen and gross energy.

TABLE 2.—*Chemical composition and gross energy value of the corn samples*

Sample of corn	Dry matter	Total nitrogen	Crude fiber	Gross energy	Sample of corn	Dry matter	Total nitrogen	Crude fiber	Gross energy
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Calories per gram</i>		<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Calories per gram</i>
Whole 1	88.73	1.67	3.80	3.967	Whole 2	89.50	1.66	3.58	4.025
Ground 1	88.99	1.67	3.89	4.033	Ground 2	88.90	1.67	3.70	4.015

The analysis of the corn samples, collected daily at feeding time for the two series of 10-day collection periods, is shown in table 2. The fineness of grinding was determined by passing portions of the ground corn through sieves of three sizes, 12 mesh (2.1 mm), 20 mesh (1.3 mm), and 40 mesh (0.64 mm). Ninety-five percent of the corn passed through the coarsest sieve, 58 percent through the intermediate sieve, and 29 percent through the finest sieve. The fineness of grinding appears to approximate the "medium ground" sample of Haberhauffe⁹ (table 1).

RESULTS OF THE EXPERIMENT

While the pigs were on the whole corn ration an attempt was made to determine what percentage of the consumed kernels appeared unbroken in the feces. By counting a 600-g portion of the corn, it was found to contain 350 kernels per 100 g. For each pig an aliquot of one-fifth of the unground feces for the collection period was carefully inspected and the unbroken kernels were separated and counted. The percentages (by number) of the consumed kernels thus recovered were 0.38 for pig 1, 1.10 for pig 3, 1.10 for pig 4, 0.38 for pig 5, and 37.90 for pig 6. The outstanding position occupied by the last animal is noteworthy, and was probably the result of defective teeth, although the truth of this supposition was not confirmed.

⁸ FORBES, E. B. A METABOLISM CRATE FOR SWINE. Ohio Agr. Expt. Sta. Circ. 152, pp. 75-85, illus. 1915.

⁹ HABERHAUFFE, W. See footnote 6.

TABLE 3.—Results of the digestion and the metabolism trials with whole and ground corn when consumed by swine

Pig no.	Body weight	Condition of corn	Daily intake in food				Coefficients of digestion				Metabolizable energy		Nitrogen balance per day
			Corn	Dry matter	Gross energy	Nitrogen	Dry matter	Gross energy	Nitrogen	Crude fiber	Total	Per kilogram of dry matter consumed	
	Kilo-grams		Grams	Grams	Calo-ries	Grams	Per-cent	Per-cent	Per-cent	Per-cent	Calo-ries	Calories	Grams
1	83.5	Whole...	1,500	1,342	6,037	25.05	85.1	82.5	65.0	68.5	4,847	3,612	+3.19
	78.9	Ground...	1,500	1,335	6,049	25.05	87.4	85.9	77.2	70.5	5,010	3,753	+4.20
2	78.5	Whole...	1,500	1,335	6,049	25.05	89.2	88.0	83.2	73.6	5,153	3,860	+5.34
	62.1	Ground...	1,300	1,153	5,157	21.71	86.0	83.6	63.0	77.0	4,174	3,620	+3.32
3	63.5	Whole...	1,300	1,156	5,219	21.71	87.6	80.3	80.0	72.9	4,367	3,778	+3.32
	67.1	Ground...	1,300	1,163	5,232	21.71	88.4	86.6	78.1	70.0	4,407	3,789	+4.01
4	64.4	Whole...	1,300	1,157	5,243	21.71	88.8	87.2	78.2	73.1	4,422	3,822	+4.28
	83.9	Ground...	1,500	1,331	5,950	25.05	91.4	89.6	80.0	(¹)	5,124	3,850	+4.31
5	86.2	Whole...	1,500	1,333	6,022	25.05	90.4	89.0	85.1	75.7	5,125	3,845	+4.11
	89.4	Ground...	1,500	1,331	5,950	25.05	82.0	80.0	71.0	46.5	4,575	3,437	+2.76
6	90.7	Whole...	1,500	1,333	6,022	25.05	87.9	86.1	78.2	70.9	5,008	3,757	+4.05

¹ This sample of feces was accidentally thrown out before the crude fiber determination was made.

The most significant results of the digestion and metabolism trials are assembled in table 3. It is evident from the coefficients of digestibility that, except for protein (nitrogen), the constituents of the ground corn were not digested to a markedly greater extent than the constituents of the unground corn. Omitting the incomplete results for pig 2, the average coefficients of digestibility of the whole corn and ground corn were, respectively, 86.6 and 88.4 for dry matter, 84.5 and 86.9 for gross energy, and 70.4 and 79.7 for protein. The percentage improvement in the digestibilities were 2.1 for dry matter, 2.8 for gross energy, and 13.2 for protein. For the four pigs for which the coefficient of digestibility of crude fiber was determined, the averages were 65.5 for the whole corn and 71.8 for the ground corn, but if the atypical results of pig 6 are omitted, the averages are very nearly the same, i. e., 71.8 and 72.2.

It is especially noteworthy that pig 6, which appeared to be passing unbroken and undigested more than a third of all whole corn kernels consumed, digested the whole corn fairly well, except for the crude fiber. While grinding improved the digestibility of corn for this pig somewhat more than for the other pigs, the relative improvement was far less than would be expected from the physical examination of the feces for unbroken kernels, proving the latter method to be quite unreliable as a criterion of completeness of digestion. The apparently unbroken kernels must have been very largely denuded of their digestible contents by the digestive enzymes.

Since corn is preeminently an energy food, the metabolizable energy values of whole and ground corn are of great practical significance. But here also the improvement brought about by grinding is slight, except for pig 6. In fact, for one pig (no. 5) no improvement at all resulted. For all five pigs upon which complete data were obtained, the metabolizable energy per kilogram of dry matter averaged 3,662 calories for the whole corn and 3,791 calories for the ground corn, representing an improvement of only 3.5 percent.

The pigs were in positive nitrogen balance in all periods. The consistently greater digestibility of the protein of the ground corn did not, however, promote consistently higher nitrogen balances, since without exception the urine from pigs on the ground corn ration contained more nitrogen than that from the same pigs on the whole corn ration. In one pig (no. 5) the lesser wastage of nitrogen in digestion on the ground corn ration was more than offset by the greater wastage in metabolism, while for another pig (no. 3) these two tendencies just balanced each other. Thus, the 13-percent improvement in the digestion of the nitrogen of the corn brought about by grinding was largely lost in metabolism, a result suggesting that the digestible nitrogen escaping digestion in the whole corn represents a fraction of the corn protein that is of low value biologically.

SUMMARY AND CONCLUSIONS

The relative digestibility of whole and ground corn and their content of metabolizable energy were determined on five pigs weighing from 135 to 196 pounds.

Grinding corn to a medium degree of fineness (29 percent passing a 40-mesh sieve, 58 percent a 20-mesh sieve, and 95 percent a 12-mesh sieve) increased the digestibility of the protein by 13 percent, but the digestibility of the gross energy of the corn was raised only 2.8 percent. The metabolizable energy was also only slightly improved; i. e., 3.5 percent. Furthermore, the appreciable advantage in protein digestibility occasioned by the grinding of corn was largely lost by greater losses of nitrogen incurred in metabolism, so that the net effect on the nitrogen balance of an animal receiving an exclusive ration of corn was slight and inconstant.

The net effect of grinding upon the nutritive value of corn for pigs of the weights used in this test is to increase its value as a source of energy by 3.5 percent. Its value as a source of protein was not appreciably altered.

STUDIES ON BEAN RUST CAUSED BY UROMYCES PHASEOLI TYPICA¹

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) rust, caused by *Uromyces phaseoli typica* Arthur, occurs in widely separated regions of the United States and has been reported from Europe, continental Asia, Japan, Australia, and other parts of the world. The occurrence of the disease in various parts of the United States is extremely sporadic as regards both regions and years. Bean rust occurs to some extent almost every year in certain States along the Atlantic seaboard and in many States in the South. In the Southeastern States and in some of the Northern States, for example, in New York and Michigan, it usually appears in the late summer or early fall after the bean crop is about matured and when the danger from severe damage is over. In Florida, on the other hand, epidemics have occurred in the late winter or early spring in such severe form that the plants were defoliated and the crop was practically ruined.

A study of the California, Idaho, and Colorado areas with respect to bean rust reveals the interesting fact that some very striking climatological and ecological differences exist. Although the climatic conditions of Colorado and Idaho seem to be quite similar, there are differences sufficient to suggest causes for the occurrence of rust epidemics in the one and not in the other State. If California is included in the comparison, there are three areas that differ in regard to the occurrence of rust—one in which epidemics occur yearly, one in which they occur occasionally, and one in which rust is never or rarely observed.

Climatological data have been collected for 3 years in the three Western States just mentioned. These data, while perhaps not explaining fully the potentialities of the different areas with respect to rust epidemics, may contribute much to an explanation of their occurrence.

In addition to observations on the epidemiology of the fungus, studies have been conducted regarding its various spore forms, their longevity and germination; the influence of temperature, humidity, and light on infection; the time required for infection; the correlation of weather conditions with rust occurrence and epidemics; physiologic forms; varietal susceptibility and resistance; and control and dissemination. This paper summarizes the results of researches carried on for about 5 years in the field and laboratory, particularly regarding phases of the problem not previously investigated or only incompletely investigated by other workers.

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² The writers are indebted to I. C. Jagger, senior pathologist of this Division, who tested the susceptibility and resistance to rust of many of the bean varieties grown at Chula Vista, Calif., over a period of 2 years.

SOURCES OF MATERIAL

Bean rust material has been collected or received from California, Colorado, Virginia, Florida, South Carolina, Tennessee, Mexico, and Australia. Spores from the various sources, except those obtained from Australia, were virulent and gave typical symptoms of the disease when used for inoculation. The collections from different areas were compared to ascertain whether there were any distinct biological or morphological forms.

EXPERIMENTAL METHODS

In studying the various phases of bean rust, such as the relation of temperature and humidity to infection, length of time required for infection, spore germination, and other phenomena, rust material from a single source was employed.

Abundant spore material was maintained by culturing the rusts on the Pinto variety in the greenhouse. This variety is a very susceptible field bean and grows well under greenhouse conditions. It was used almost entirely except where variety tests were being conducted or where a differential host was desired. The Tennessee Green Pod, another very susceptible variety, was used in a few experiments.

In order to have at hand an abundance of rust material, plants were inoculated about every 7 to 10 days by atomizing them with urediospores suspended in water or by spreading them on the leaf with a camel's-hair brush. The plants were then confined in an infection chamber held nearly at saturation and at a temperature of about 18° to 20° C. for 15 to 24 hours. The temperature varied somewhat with the season, but the best results were obtained when it was not above 21°. The minute, pale-colored flecks beneath the epidermis were the first evidence of infection under greenhouse conditions and appeared in about 4 or 5 days. At the end of about 10 days the pustules opened.

SPORE FORMS

LIFE CYCLE

The life cycle of the bean rust in the greenhouse calls for particular mention. Andrus (1)³ has previously reported on observations of the aecial and spermatogonial stages in the development of the bean and cowpea rust. *Uromyces phaseoli typica* forms teliospores abundantly in the fall on beans in Colorado, and material from there as well as from California has been employed for most of the experimental work. If field-collected teliospores are germinated and then placed on bean leaves in the greenhouse and given suitable temperature and humidity conditions, fertile aecia are formed, followed after reinfection by the production of uredia and teliosori. On the other hand, a limited number of tests have indicated that if teliospores produced in the greenhouse are germinated and placed on the leaves under conditions similar to those just described, only sterile aecia are produced, even though the nectar from different aecia is mixed. Successful infection with greenhouse-produced teliospores was ob-

³ Reference is made by number (italics) to Literature Cited, p. 758.

tained on only one occasion. In this case the several aecial infections all proved to be of the same sex and remained sterile after transfer of spermatia. Because of this fact and the limited number of tests made, it cannot be said that the complete life cycle of the bean rust has been reproduced under greenhouse conditions. It would seem that if a beginning is made with teliospores of the proper origin the full life cycle of the bean rust is possible under greenhouse conditions.

TELIOspore FORMATION

Observations have been made on teliospore production under greenhouse conditions and on the variation in the ability of cultures of different origin to produce teliospores. Bean rust has been collected from various parts of the United States, and three areas—southern California, Colorado, and Virginia—may be selected to illustrate the differences in teliospore formation. In both Colorado and Virginia teliospores develop abundantly in the latter part of the summer before the vines have been killed by frost. On the other hand, they rarely form in southern California, although the vines are killed by frost during the winter months. In an abundance of material from there, only 2 or 3 pustules containing teliospores have been found; the strain seems to have almost lost the power to form teliospores. Out of many inoculations made in the greenhouse with spores from California, teliospores have never developed, while the production of such spores on plants inoculated with material from Colorado or Virginia is of common occurrence.

In tropical and subtropical regions the preponderance of uredia of different rust species has frequently been noticed and has sometimes been attributed to the lack of recurring conditions that retard growth. Many theories have been advocated to explain this phenomenon. Ivanov (15) found that cool temperatures checked urediospore production and increased the development of teliospores, while Smith (28) concluded that abundance of soil moisture stimulated the development of the host and increased its resistance, as a result of which the fungus (*Puccinia asparagi* DC.) was retarded and forced to develop telia. These results are contrary to those obtained by Sheldon (26, 27), Stakman and Levine (29), Ward (31), Arthur (2), and others, who maintain that, as a rule, whatever favors the development of the host also favors the rust. In 1928 Waters (32) carried out an elaborate series of experiments designed to study the influence of environmental factors on the production of teliospores and urediospores on a number of hosts, including beans, and concluded that all rusts studied are directly dependent upon photosynthetic activity of the host. He states (32, p. 209):

Any single factor or set of factors, such as light, temperature, and moisture, or, as in the case of climate, a complex of these factors may so influence and do influence the metabolism of the host, that the fungus reacts by changing from the uredinial to the telial generation, or, under proper manipulation, in the reverse direction.

To the writers the explanation quoted above does not seem entirely adequate. Bean rusts from various sources have been propagated in the greenhouse for about 5 years and under a considerable diversity of conditions. Those originating in Colorado and Virginia, where teliospores normally develop, produced them abundantly in the greenhouse. On the other hand, the strain from California has

consistently failed to produce teliospores under any of the conditions to which it has been exposed. One of the usual concepts regarding the behavior of rusts is that teliospore formation is more or less coincident with the aging of the plant and the mobilization of food materials for the production of spores (3). Measured by these standards, the bean rust should develop teliospores in California and in the greenhouse as well as do the rusts from other sources. In a consideration of these rusts under various conditions one cannot ascribe to environment alone the difference in their behavior in respect to teliospore formation.

Gassner (12) observed differences in the time of teliospore formation among several species of *Puccinia* on cereals. Withholding mineral nutrients from the host and mutilating the host tissue failed to hasten the production of teliospores.

Raines (25) suggested that the protoplasmic constitution of the fungus may be a factor in teliospore production. In experiments conducted by him he was able to show a variation on the part of *Puccinia coronata* Cda. (referred to by him as *P. coronifera* Kleb.) in the production of teliospores. By selecting spores from pustules containing no teliospores he was able to decrease the number of teliospores from pustules; likewise by selecting spores from pustules containing teliospores he increased the number of such pustules. In view of these results, it is conceivable that a strain of rust might be developed which had entirely lost its power to form teliospores. It is not improbable that the rust in southern California, having been propagated naturally for a number of generations from the urediospores, has lost its ability to produce teliospores. The winter season is short, so the urediospores would have to survive only a few weeks to carry the rust from one crop season to the next.

In an attempt to stimulate teliospore production, the methods employed by Waters (32) were tried. From the results obtained by the writers there are reasons to believe that there are at least two forms of bean rust that differ genetically in the ability to form teliospores at some stage of their development. Within the teliospore-producing strain the abundance of teliospores as well as the time when they appear may be controlled to an appreciable extent by altering host metabolism.

LONGEVITY OF UREDIOSPORES AND TELIOSPORES

A study of the longevity of rust spores has shown that the urediospores of some rusts will retain their viability for several months and in some cases for a year, and the teliospores for a longer time. The evidence contributed by many investigators indicates that the viability of the spores is closely correlated with the temperature and humidity at which they are stored. In this connection it is interesting to note the results of Peltier (23), who found that the urediospores of *Puccinia graminis tritici* Eriks. and Henn., form III, when stored at humidities varying from 38 to 70 percent and at temperatures of 5° to 15° C., retained their viability better than when stored at higher temperatures. The viability was reduced appreciably when the spores were stored at high and low humidities. On the other hand Bailey (4) found a humidity of 20 to 40 percent at a temperature of 23° to be the optimum for the storage of urediospores of *P. helianthi* Schw., which retained their viability for 185 days under such condi-

tions. Hart (14) found storage of *Melampsora lini* (Pers.) Lév. spores at a relative humidity of 40 percent and a temperature of 7° to be the most favorable for longevity. Lambert (19) showed that the teliospores of wheat rust usually lose their viability a few months after maturity, but if kept in cold storage their longevity may be prolonged for nearly a year.

The investigations conducted by the writers on the longevity of bean rust spores are not intended to be complete. They were designed primarily to determine whether the spores were capable of maintaining their viability for a considerable length of time under what might be regarded as favorable conditions, and not what effect different temperature and humidity conditions would have on their longevity.

In October 1931, bean leaves with abundant rust pustules containing both urediospores and teliospores were collected and stored in an incubator at 9° C. with the relative humidity at approximately 73 percent. This experiment was intended to show the longevity of urediospores under known conditions and not the longevity of the teliospores, which were previously shown to germinate for a number of months after maturity. However, teliospores were later germinated on drops of water on cover slips in Petri dishes at intervals of 2 weeks. This method was found suitable for the purpose. Each sample was kept under observation for about 3 weeks. From 50 to 60 percent of the teliospores had germinated at the end of 207 days, after which no more tests were made.

The longevity and viability of the urediospores were determined by a somewhat different method. Instead of testing spore germination in water on slides, the spores were used to inoculate bean plants in the greenhouse. After an initial storage period of 10 weeks, young plants of the Pinto variety were subjected to infection at intervals of 2 weeks, with the result that a few scattered infections were obtained at the end of 26 weeks, but none after 28 weeks. These results indicate that bean rust might be carried over from one season to the next by means of the urediospores.

COMPARISON OF BEAN, COWPEA, AND STROPHOSTYLES RUSTS

The bean rust and the cowpea (*Vigna sinensis* (L.) Endl.) rust were at one time thought to be identical. Fromme (7) later proved them to be distinct pathogenetically; he also proved that there were morphological differences, from which he concluded that the cowpea rust was a clearly distinct species and assigned it to *Uromyces vignae* Barcl. A new description of the species was appended. The work of Fromme was so complete as to leave little doubt of the correctness of his conclusions. In view of that fact the discussion of the cowpea rust will be confined to some additional evidence acquired in connection with the investigations of bean rust.

In 1929 badly rusted Blackeye cowpeas were found growing among severely rusted beans in California, which suggested that the two rusts might be identical. A series of cross inoculations were made in which it was found that bean rust collected from widely separated regions would not infect cowpeas and that cowpea rust would not infect beans. Other differences were also noticed. The teliospores of cowpea rust germinate readily soon after maturity, whereas the bean rust teliospores require a rest period or germinate only sparingly

and feebly when first matured. The aecia of both have been produced in the greenhouse. Aecia of bean rust are white; those of cowpea rust are yellow. Likewise the nectar of the bean rust spermogonia is white, and that of the cowpea rust spermogonia is yellow. Morphological differences in the teliospores pointed out by Fromme were also found.

A rust with some points of resemblance to both bean and cowpea rusts was found on *Strophostyles helvola* (L.) Britt. in Virginia, not far from Washington, D. C., which might be referred either to *Uromyces phaseoli typica* or to *U. phaseoli vignae* (Barcl.) Arthur. Teliospores in considerable abundance were collected, and attempts were made to infect beans and cowpeas, but without success. Conversely, *S. helvola* could not be infected with rust from beans or cowpeas. It was difficult to propagate *S. helvola* in the greenhouse, and only a few plants were available for inoculation. In view of the limited number of inoculations, the results are to be interpreted as merely suggestive. Cowpeas and beans, however, were available in abundance for inoculation with the *Strophostyles* rust. Aecia of this rust were developed on *Strophostyles* in the greenhouse by inoculation with sporidia from teliospores. The nectar and the aecia are yellow, and in this respect *Strophostyles* rust resembles the cowpea rust. On the other hand, *Strophostyles* rust resembles bean rust in that a considerable rest period is required before the teliospores germinate readily. *S. helvola* was not readily infected in the greenhouse with rust spores from *Strophostyles* collected in the field. The aecia were formed, but no other form of rust developed. On the other hand, the cowpea and bean rusts produce all spore forms under greenhouse conditions. The writers are inclined to agree with Fromme that *Strophostyles* rust probably represents a different species from that found on either beans or cowpeas.

ENVIRONMENTAL FACTORS

RELATION OF TEMPERATURE TO SPORE GERMINATION

The urediospore seems to be the principal spore form involved in the dissemination of the bean rust and in the damage to the crop that follows. Bean rust occurs at different seasons of the year in regions differing considerably in temperature and subject to wide temperature fluctuations, which suggests that there is some correlation between these factors and the presence of rust and rust epidemics. Temperature alone, of course, is not the limiting factor for infection, since there is also a minimum of atmospheric moisture that will permit spore germination. In case a high humidity persists for only a few hours during a day, it is highly essential that the spores germinate in the briefest time possible if infection is to be established. With this thought in mind a series of experiments was designed to determine the time required for spore germination, the minimum moisture requirement, the optimum temperature, and the temperature range.

The urediospores of the bean rust will germinate as soon as they are mature. It has been noted that they may deteriorate in a short time after maturity and that better infection may be obtained if the spores are taken from freshly opened pustules than if collected from those several weeks old. On the other hand, if they are properly manipulated their virulence may be maintained for a considerable

length of time, as shown (p. 741). The germination experiments were made with spores 1 to 2 weeks old taken directly from the pustules on the leaves. They were sprinkled thinly on a 2-percent potato-dextrose agar in Petri dishes and placed in incubators with temperatures ranging from 0° to 36° C.

The inability to duplicate in the laboratory the conditions that surround the spores in nature has frequently aroused considerable controversy about the interpretation of the results. The germination of the spores under artificial conditions cannot be interpreted as representing exactly what takes place under natural conditions. With this fact in mind, germination tests with bean rust spores were made with the hope that some data might be obtained that would help to explain the occurrence of bean rust in regions where it does not appear annually in an epidemic form.

Figure 1 illustrates the germination of urediospores during a period of 24 hours in controlled temperature chambers. In a consideration

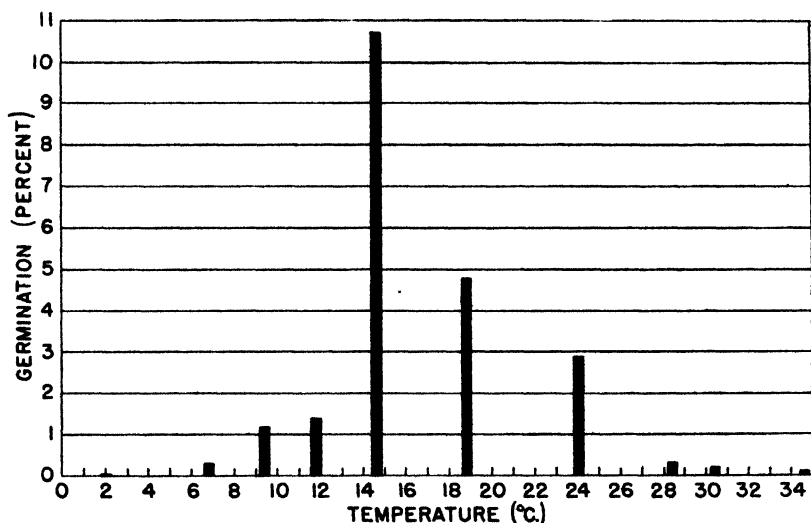


FIGURE 1.—Approximate hourly average percentage of urediospores of *Uromyces phaseoli typica* germinating during a period of 24 hours at constant temperatures.

of these results it must be kept in mind that the optimum temperature for spore germination was considered to be that at which the largest percentage of spores germinated in a fixed period, in this case 24 hours. The germ tubes were longer on an average at the temperatures where the largest percentage of germination took place.

The results show that the percentage of spore germination was not large at any temperature. The percentages of germination were arrived at by the following method: The plates were examined every hour and a count was made of the total number of spores and number of spores that germinated in five different fields under the microscope and averaged. The counts in many cases amounted to several hundred spores. At temperatures of 12°, 15°, and 17° C., spore germination was evident in 2 hours and increased with the length of time. In 4 hours germination had taken place over a range of temperatures from 10° to 31°, and in 5 hours from 7° to 35°.

From figure 1 it is seen that the optimum temperature for the germination of the urediospores, using the percentage of germination at the end of a given time as a criterion, is approximately 14.5° C. Above that optimum the percentage of spore germination decreases rapidly. The minimum was found to be 1.8° and the maximum slightly above 34.5°.

RELATION OF TEMPERATURE TO INFECTION

No attempt was made to determine exactly the maximum and minimum temperatures for infection. The optimum was found to be approximately 17.0° C., which is somewhat higher than the optimum for spore germination. Above and below that temperature the percentage of infection decreased. At 27° no infection was obtained. In studying the lower range for infection, difficulties were encountered because the plants exhibited considerable injury from chilling. The few results obtained indicate that infection would probably take place at a temperature as low as the plant could endure without injury.

RELATION OF HUMIDITY TO INFECTION

The rapidity with which the spores will germinate and infect the host is of primary importance, especially in arid regions where the relative humidity is high enough for spore germination to take place for only a short time during any 24-hour period, which is usually at night. Frequently the humidity is high for a day or two during rainy periods, which rarely occur in the arid regions of the West. The probability is that, in order to cause infection in a dry climate, a rust spore must germinate and infect the host within a few hours.

The results of experiments on the relation of humidity to infection by the bean rust show that within the range of temperatures permitting spore germination infection took place readily when the plants were exposed to a relative humidity of 96 percent or higher. At a relative humidity of 95 percent the percentage of infection was somewhat reduced, and at lower humidities no infection took place, irrespective of time. These results agree closely with those obtained by Fromme (6), Lauritzen (21), and others.

Data were likewise obtained on the drying of the foliage after atomizing with rust spores and on the effects of air movement on infection. It was found that infection readily took place when the moisture was allowed to evaporate from the leaves immediately after the application of spores to the plants, provided the plants were placed in an infection chamber with a saturated atmosphere. If, however, the dried plants were subjected to a relative humidity of 96 percent, no infection resulted or the amount was considerably reduced. An examination of plants held under these two conditions showed that in the one case moisture had condensed on the foliage and in the other it had not.

If plants are confined in a chamber at a relative humidity of 96 percent, with moisture already present on the foliage, it is possible for infection to take place before the droplets on the leaves have evaporated. Air movement also plays a part here. The above results were obtained without any artificial air movement. If the air is kept in circulation by means of a small fan blowing over the foliage, no infection takes place at 96 percent relative humidity. This can be

explained by the fact that the moisture on the leaves has evaporated before penetration takes place. On the other hand, in an atmosphere of 98 percent relative humidity or higher, air movement did not reduce the amount of infection.

RELATION OF LIGHT TO INFECTION

The foregoing experiments, designed for another purpose, did not lend themselves readily to a thorough investigation of the effect of light and high humidity on infection. A series of experiments was therefore designed in which young, vigorously growing plants were atomized with spores suspended in water and immediately placed in the infection chamber with a saturated atmosphere and subdued light. At daily intervals for a period of 6 days, several pots containing three plants each were removed from the infection chamber. Attempts were made to compare the intensity of the light within the chamber with that outside, but no satisfactory method was found, so this undertaking was finally abandoned. The infection chamber was placed in the greenhouse so that the temperature within it would approximate that on the outside where the plants were placed after their removal. In one series of experiments the door of the infection chamber was opened 24 hours after inoculation so that the humidity within would be about the same as that outside, where the plants were to be placed at the end of each removal period. In another experiment the door of the infection chamber was kept closed and the humidity high, and the plants were removed from it at intervals to another chamber differing only in being uncovered and thereby supplied with the normal amount of light.

Without detailing the results of these several series of experiments, it may be stated that they all pointed to the one general conclusion—that any abnormality in the functioning of the host is accompanied by delayed or reduced infection. Although normal infection was the rule after an exposure for 24 to 48 hours in an infection chamber, regardless of the light intensity, beyond that length of time not only was the percentage of infection reduced but the development of the pustules was noticeably retarded.

On plants removed from the dark infection chamber after 72 hours pustules developed about 24 hours later than on plants removed after 48 hours. In general this was true for each succeeding 24-hour period up to the end of 6 days, when the experiment was terminated. After about 48 hours in the infection chamber the plants began to show the results of this unfavorable environment by a slight yellowing of the foliage which became more pronounced as time went on. At the end of 6 days the plants were badly etiolated and some were wilted. Plants kept in the chambers for 5 or 6 days rarely recovered, and the percentage and rate of development of the rust pustules were correspondingly reduced. Plants confined for a shorter time in an environment of reduced light usually recovered, but the development of the sorus was correspondingly delayed. These data indicate, as other investigators have pointed out, that an environment unfavorable to the host is likewise unfavorable to the rust parasite.

TIME REQUIRED FOR INFECTION

Since humidity is vital to the germination of the spores and infection of the plant, its relation to the time required for germination and for infection to take place becomes increasingly important. In general, under field conditions there are comparatively few consecutive hours with a humidity of 95 percent or more, as figures 2, 3, and 4 show. (These charts, showing striking differences in climatic behavior, were selected as typical of the regions during the bean-growing season when rust infection would normally take place.) The fewer the hours required for infection the better is the chance for the rust to become established. In certain regions rains are frequent and dews are of almost daily occurrence, both of which contribute to the ease of establishing the disease. In the case of either dews or rains moisture would probably be deposited on the foliage for a sufficient length of time to permit germination of the rust spores.

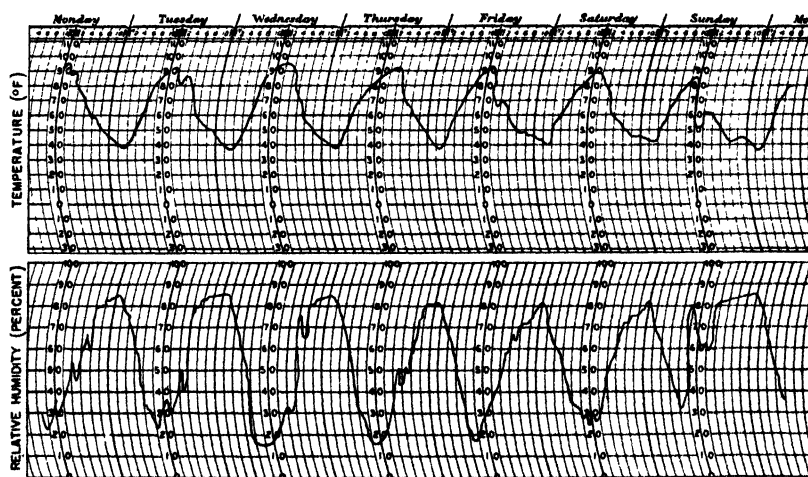


FIGURE 2.—Temperature and relative humidity at Greeley, Colo., over a period of 1 week, typical of the area during the bean-growing season when rust infections would normally take place

On the other hand, in Colorado, for example, rains are infrequent and dews are rare, and the humidity is relatively low except for a few hours at night. It is in such regions that the outbreak of bean rust in an epidemic form cannot so easily be explained.

Controlled experiments conducted in an infection chamber under greenhouse conditions have been employed to determine the time required for infection to take place. The humidity of the infection chamber was held as near to saturation as possible, and in practically all cases there was a deposit of moisture on the foliage of the plants and on the walls of the infection chamber. The temperature varied somewhat but was held within the range favorable for infection. Vigorously growing young Pinto bean plants were atomized on both the upper and lower surface of the leaves with a water suspension of spores taken from young rust pustules. They were immediately placed in the infection chamber, which was covered in order to reduce the intensity of the sunlight. In duplicate experiments, two pots

with 2 to 4 plants each were removed at definite intervals from the infection chamber and placed in the greenhouse, where the temperature was approximately that of the infection chamber. The relative humidity of the greenhouse varied from 47 to 63 percent, so that the leaves dried in about 10 to 15 minutes after removal.

The results showed that no infection took place on plants removed from the infection chamber 2 to 6 hours after inoculation. Only very few pustules were formed on plants held in the infection chamber for 8 hours, but there was an appreciable increase at 10 hours. When the plants were in the chamber 12 to 18 hours infection was severe, and the results indicate that this length of time represented about the optimum. An important point in connection with the data is that under favorable temperature conditions some slight infection takes place within 8 hours.

It was noted also that after confinement of the plants in the infection chamber for 20 hours or more the percentage of infection was less

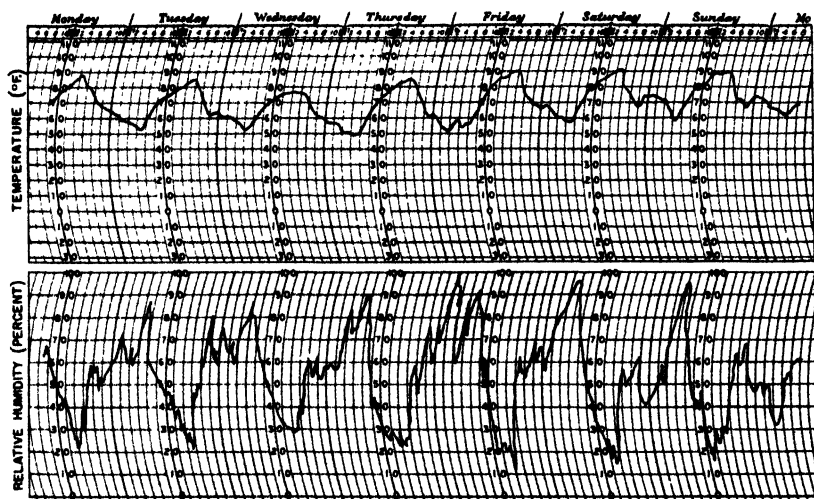


FIGURE 3.—Temperature and relative humidity at Twin Falls, Idaho, over a period of 1 week, typical of the area during the bean-growing season when rust infections would normally take place.

than after confinement for 12 to 18 hours. The decrease in the percentage of germination on plants confined in infection chambers for more than 18 hours is probably closely correlated with the condition of the plant.

Arthur (2), Ward (30), Raines (25), Stakman and Levine (29), Peltier (24), and others have emphasized the fact that there is a close relation between the vigor of the host and rust infection of cereals. In our experiments the plants were kept in subdued light for the entire length of the incubation period and in an almost saturated atmosphere, both conditions being abnormal. In consequence the reduced photosynthetic activities that prevailed were reflected in the vigor of the plant, and with this was associated a reduction in the percentage of infections.

CORRELATION OF WEATHER CONDITIONS WITH RUST OCCURRENCE AND EPIDEMICS

The bean rust epidemic in the Greeley, Colo., section in 1927 was the most severe that has been reported for the bean crop. Almost all the susceptible varieties were entirely destroyed, and those that were only partially resistant were badly damaged. On the other hand, no reports are known of rust ever occurring in southern Idaho. The situation in Colorado and Idaho is in sharp contrast to that in the coastal section of southern California, where rust epidemics occur every year. The question naturally arises, What are the conditions that result in a rust epidemic occasionally in Colorado, never in Idaho, and yearly in southern California? Probably because of the many factors involved, the question cannot be answered with certainty, but both temperature and humidity doubtless play important parts. Any data collected are seemingly too incomplete to account

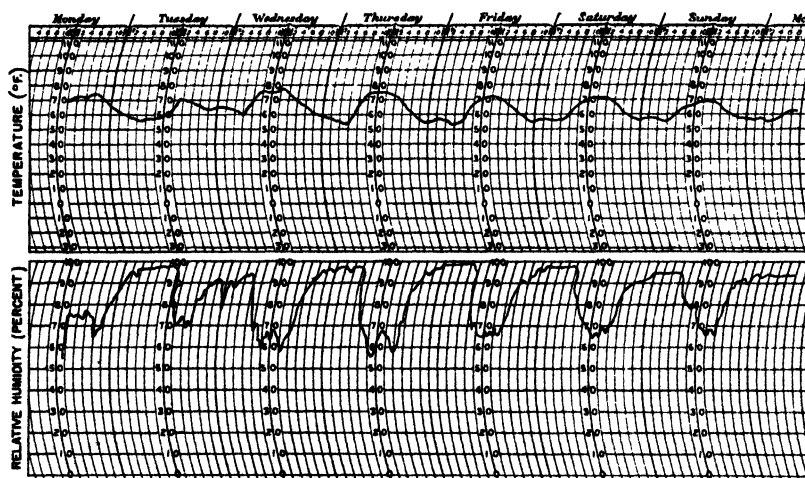


FIGURE 4.—Temperature and relative humidity at Chula Vista, Calif., over a period of 1 week, typical of the area during the bean-growing season when rust infections would normally take place.

for the exceptions and variations from season to season and between regions widely separated geographically. The source of the inoculum, the condition of the host, and favorable temperature and humidity for germination of the spores are doubtless important factors that contribute to the initial infection. Conditions favorable to the production of a second and probably a third generation of spores would perhaps be required to multiply them in sufficient numbers to create an epidemic. Experiments have shown that 8 to 10 days are required from the time of inoculation for urediospores to mature. On this basis about 3 weeks would be required to spread the infection sufficiently to constitute an epidemic. If, however, the proper environmental conditions, such as temperature and humidity, did not coordinate with spore dissemination, succeeding generations would fail and no epidemic would materialize. The conclusion is probably warranted that during those years when no rust occurs or when it occurs only to a limited extent the chain of favorable conditions for

multiplication and dissemination of the spores is broken sometime during the summer, before the infection reaches the epidemic stage.

With the hope of obtaining some information on the epidemiology of bean rust, constant temperature and humidity records were taken during the summer months of 1929 and 1930 at Greeley, Colo. (fig. 2), Twin Falls, Idaho (fig. 3), and Chula Vista, Calif. (fig. 4). As inaccurate as such records conceivably may be, certain facts are revealed that may contribute to a partial explanation of why the three areas differ so markedly with respect to the occurrence of bean rust.

Controlled experiments have shown that the urediospores of the bean rust infect best at a temperature of about 17° C. It was also demonstrated by similar experiments that no infection takes place at a humidity below 95 percent, the optimum being near saturation. To insure infection it was necessary to hold the plants under these conditions for at least 8 hours, and from 12 to 18 hours or longer were required for optimum results. With these facts in mind, it is interesting to compare the three hydrothermographic chart records (figs. 2, 3, and 4) with respect to daily temperatures and relative humidities. These charts record the results for only a single week, but they were selected from a large number as typical of the three areas and indicate differences that are more or less constant.

It will be seen that in southern California (fig. 4) the proper temperature and humidity requirements for spore germination and for infection occur daily. At night the humidity approximates saturation for 8 to 12 hours, and the temperature is at the optimum for rust infection. A fog regularly sweeps in from the sea during the night and often remains until 9 or 10 a. m., and during this time the foliage is wet. Under such ideal conditions bean rust infection occurs regularly every year, even though rains rarely occur during the summer months. Quite different conditions prevail in southern Idaho (fig. 3), except that, as in southern California, summer rains are rare. The temperature is high in the daytime, but at night it is within the optimum for infection. Figure 3 shows that, while the relative humidity may reach 95 percent or more for a very short time during the night, it does not last long enough to allow the spores to germinate and infection to take place. An analysis of figure 3 with respect to temperature and relative humidity would suggest that bean rust would probably never become established in southern Idaho.

The record for Greeley, Colo., falls between those of Chula Vista, Calif., and Twin Falls, Idaho. Rain may occur at any time during the growing season, although long rainless periods occur every summer. The usual night temperatures are optimum for infection, but the relative humidity is extremely variable at night and usually is not high enough to permit infection, although it may occasionally occur. Such high humidities might be expected now and then to correspond in the matter of time with the dissemination of the rust spores. The occasional high humidities, together with rainy periods lasting several days and coming at just the proper time, might account for the occasional epidemics of bean rust in that area.

The absence of a primary source of spore material can hardly be regarded as a limiting factor. Neither can the abundance of spore material insure a rust epidemic the following year. It is the practice in Colorado to stack the bean straw after threshing, feed it to sheep,

or scatter it on the fields. In 1928, after the epidemic of 1927, abundant spore material was found in the straw stacks throughout the area. Teliospores were abundant in the straw and were viable into the summer of 1928. Yet in 1928 there was only a slight amount of rust, distributed more or less locally, which suggests that the proper environmental conditions did not occur or that they were not correlated with the generations of rust development.

VARIETAL SUSCEPTIBILITY AND RESISTANCE

Data on the susceptibility of beans to rust are contained in publications by Fromme and Wingard (8, 9), Morse,⁴ Gassner (11), and Jordi (16). Fromme and Wingard inoculated in the field and greenhouse a large collection of varieties and determined their relative susceptibility by using the Tennessee Green Pod, one of the very susceptible varieties, as a standard for comparison. Morse lists a number of varieties with respect to their susceptibility to rust in Maine. Gassner carried out inoculations in South America and found considerable difference in the susceptibility of varieties. Jordi took field notes on 5 pole varieties in Europe and found 4 to be very susceptible and 1 quite resistant.

Fromme and Wingard adopted the scheme of grading the Tennessee Green Pod 100 percent susceptible and comparing other varieties with it. Those showing greater susceptibility than the Tennessee Green Pod were graded proportionately above 100, and those less susceptible were graded correspondingly less.

In the course of investigations of bean rust and other bean diseases the writers had occasion to make a large collection of domestic and foreign varieties with the hope that some of them might be of suitable type and show sufficient resistance to rust and other diseases to be used as parents in the production of resistant strains. Many lots of seed were received from China, Union of Soviet Socialist Republics, Germany, Mexico, Chile, Venezuela, Rhodesia, Uganda, Puerto Rico, Iran, Japan, and Guatemala, through the Division of Plant Exploration and Introduction, Bureau of Plant Industry, United States Department of Agriculture. Most of these varieties were unidentified as to name but were designated by a serial number. Many lots were received independently from foreign countries and were often mixed and unnamed. All these were tested for rust resistance. The experiments on susceptibility of most of the domestic varieties were carried out in two localities, namely, in the greenhouse at Washington, D. C., and in the field at Chula Vista, Calif., where rust epidemics occur annually. While artificial inoculation was practiced once or twice in the field tests, it was not generally necessary. Since the season in California is sufficiently long to grow two crops, one test was made in the spring and one in the fall. Greater infection occurred in the fall than in the spring. The data from the field and greenhouse experiments are shown in table 1.

Many of the varieties tested by Fromme and Wingard were included in the present collection. The results on the whole agreed very closely, although their tests were made under somewhat different climatic conditions.

⁴ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. RUST CAUSED BY *UROMYCES APPENDICULATUS*. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bull. 2:174. 1918. [Mimeographed.]

Another system of grading resistance and susceptibility has been employed, that of Jordi, which consists in arranging the varieties in a scale from 0 to 10, immune varieties being graded 0, the most susceptible 10, and intermediate forms between these two extremes. The Kentucky Wonder was used in California as a standard of comparison because of its extreme susceptibility to rust. On the other hand, the Pinto was used mostly in the greenhouse tests at Washington, D. C.

The susceptibility or resistance of the varieties from foreign sources was determined by greenhouse inoculation. Where it was possible to segregate the mixtures according to color, shape, and other characters of the seed, infection experiments were conducted. In cases where the samples could not be identified with certainty, the results are not recorded in the table. Suffice it to say that practically all the unidentified foreign lots showed some degree of susceptibility.

TABLE 1.—*Bean varieties arranged according to degree of rust susceptibility under field conditions in southern California, together with infection ratings in greenhouse tests at Washington, D. C.*

Group, susceptibility, and variety	Infection rating ¹		Group, susceptibility, and variety	Infection rating ¹	
	Field results ²	Greenhouse results ³		Field results ²	Greenhouse results ³
Field beans.			Garden Beans—Continued		
Very susceptible			Slightly susceptible:		
California Pink	10	9	Burpee Brittle Wax	3	2
California Red	10	9	Burpee White Wax	3	—
Pinto	10	10	Fordhook Favorite	2	2
Red Mexican	10	9	French Horticultural	2	2
Blue Pod Small White	9-10	9	Improved Kidney Wax	2	3
Large White	9-10	9	Low Champion	2	2
Great Northern	7-10	9	Rust Proof Golden Wax	2	—
Michigan Pea	7-9	8	Late Stringless Refugee	2	2
Moderately susceptible			Keeney Rustless Wax	1-2	1
Genuine Small White	6-8	9	Hodson Wax	0-4	2
Tepary (<i>Phaseolus acutifolius latifolius</i> Freeman)	6-7	5-10	Longfellow	0-4	5
Robust	5	9	Red Valentine	0-4	7
Slightly susceptible:			Bountiful	0-3	4
Bayo	2-4	5	Black Valentine	0-3	3
White Kidney	3	3-8	Burpee Stringless Green	—	—
Brown Swedish	2-3	—	Pod	0-3	4
Red Kidney	2-3	7	Currie Rust Proof Wax	0-3	2
Yellow Eye	2	—	Full Measure	0-3	5
Large White Marrow	1-2	7	Pencil Pod Black Wax	0-3	3
Cranberry	0	0	Refugee Wax	0-3	—
Garden beans:			Wardwell Kidney Wax	0-3	3
Very susceptible:			Black Wax	0-2	3
Kentucky Wonder (brown-seeded)	10	10	Davis White Wax	0-2	7
McCaslan	8-10	—	Giant Stringless Green	—	—
Tennessee Green Pod	7-10	10	Pod	0-2	3
Moderately susceptible:			King Mammoth Horticultural	0-2	2
Striped Greasback (Scotia)	6-8	9	Refugee 1000-1	0-2	2
Golden Cluster Wax	6-7	4-7	Sure Crop Wax	0-2	3
Kentucky Wonder (white-seeded)	5-7	3-9	Canadian Wonder	0-1	7
Kentucky Wonder Wax	6	2-0	Low Champion Bush	0-1	—
Dutch Case Knife	4-6	9	Improved Golden Wax	0-1	3
			Long Yellow Six Weeks	0-1	2
			Mohawk	0-1	2
			Refugee (extra early)	0-1	2
			Round Pod Kidney Wax	0-1	2
			Lazy Wife	0	1
			Weber Wax	—	1

¹ On scale of 1 to 10.

² Brown-seeded Kentucky Wonder, graded 10, used as standard of comparison. Notes by I. C. Jagger.

³ Pinto, graded 10, used as a standard of comparison. Notes by C. F. Andrus.

In general there was appreciable injury to the varieties when the rust reaction was scored 5 to 10, but little or none when scored below 5. Probably varieties scored below 5 would be expected to suffer from rust only when exposed to very severe and heavy infection. A score of 1 indicates that a trace of rust was perceptible only on close examination. It has been found that the most resistant varieties may be expected to show a trace of rust under very favorable conditions.

A careful examination of table 1 shows that, out of a total of 61 varieties listed, only 19 show under field results a degree of susceptibility that would be regarded as positively destructive; 7 of these are pole varieties, 11 field beans, and 1 (Tennessee Green Pod) a bush bean.

The percentage of highly susceptible varieties among the so-called field and pole beans (field results) is relatively high. Among the pole beans only 2 varieties (Lazy Wife and King Mammoth Horticultural) showed any marked degree of resistance, while 7 out of 18 field bean varieties would be considered sufficiently resistant to recommend for growing in localities where rust epidemics occur.

Twelve varieties of lima bean (*Phaseolus lunatus macrocarpus* Benth.) grown in the United States and obtained from commercial sources, 1 variety from China, and 2 from Japan were tested for rust resistance by inoculation in the greenhouse, with the following results:

Variety ⁵	Rating
Carpenteria.....	8
Challenger.....	8
Dreer.....	8
Early Leviathan.....	8
Emerald Isle.....	8
Evergreen.....	8
Fordhook.....	8
Henderson.....	2
King of the Garden.....	8
Sieberts.....	8
Sieva.....	2
Wood Prolific.....	2
28805 (from Japan) ⁶	3
28807 (from Japan) ⁶	3
29067 (from China) ⁶	2

All the domestic varieties except 3 (Wood Prolific, Sieva, and Henderson) are graded 8 in susceptibility and therefore fall in the class of very susceptible varieties. Considerable damage to most of the varieties might be expected when they are grown in localities where rust is likely to develop. The three lots or varieties from foreign sources showed considerable resistance to rust, as their ratings of 2 and 3 indicate.

PHYSIOLOGIC FORMS

The investigations on bean rust indicated a considerable difference in the relative susceptibility and resistance of different varieties. Some were extremely susceptible, and urediospores were abundantly formed in the sori. In the case of susceptible varieties a ring of secondary sori, and frequently even a third ring, develops outside the

⁵ The seed of the 12 domestic varieties was obtained from commercial sources. The writers have used the trade names without regard to possible synonymy.

⁶ Seed obtained from D. N. Shoemaker, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.

primary sorus, so that a spot of considerable diameter finally results on both the upper and lower surfaces of the leaf. On the other hand, in partially or almost completely resistant varieties, abortive sori, their size and extent depending on the degree of resistance of the host, are produced, in which few or no spores develop. Such abortive sori are often almost invisible to the unaided eye. The fungus penetrates the host tissue generally, growing through the leaf and forming a lesion on both the upper and the lower surface. The epidermal cells are killed and collapse, so that a slightly sunken area is formed on the surface. In some cases the infection spots suggest a corrosion of the epidermis with later collapse of the epidermal cells. A few spores may be formed on the lower leaf surface but rarely in the dorsal lesions.

In testing the resistance and susceptibility of beans to rust several German varieties of the Kentucky Wonder type were inoculated with a rust from southern California and with one collected in the vicinity of Washington, D. C. Only sterile abortive flecks developed on some of these varieties, showing that they possessed considerable resistance. However, a few scattered, normal, sporulating sori were found on practically all the plants of the resistant varieties. These sori were typical of those found on plants of susceptible varieties, from which it was concluded that two physiological forms of the bean rust composed the original culture, one being present in an extremely small proportion. For the sake of clarity the parent culture was designated form 1 and the rare culture form 2. Form 2 was isolated by the inoculation of additional plants of the same varieties with spores from the normal sori, and the results were compared with the results of the parallel inoculations with form 1. Form 2 gave good infection, with the production of normal spore-producing sori, while form 1 gave the usual flecking and a few normal sori, which persisted probably as a contamination of the parent culture. These experiments were repeated several times with the result that the difference in virulence of the two forms was maintained throughout.

Form 2 was isolated from bean rust material from two widely separated places—California and Washington, D. C. The two original cultures are pathogenetically alike when inoculated on the same varieties of beans, but there are reasons to believe that they differ genetically. The rust of California origin does not include the telial stage and therefore must have been without sexual reproduction for at least many generations. On the other hand the rust culture collected in the East normally produces telia both in the field and under greenhouse conditions. It appears, therefore, that both forms 1 and 2 are represented by teliospore-producing and non-teliospore-producing strains. In other respects the two forms appear to be morphologically alike.

The data recorded in table 2 call for some explanation. Kentucky Wonder Wax, white-seeded Kentucky Wonder, and brown-seeded Kentucky Wonder are domestic varieties and are cultivated extensively in different sections of the United States. The green-podded Kentucky Wonder varieties grown in the United States are quite susceptible to rust; although the one with white seed was claimed to be resistant in California, it was found not to be when subjected experimentally to rust infection. On the other hand the Kentucky Wonder Wax was extremely variable, some plants being almost immune to form 1, while others were susceptible. All plants were very sus-

ceptible to form 2. The data from this variety would indicate that it is not a pure line, or at least not entirely fixed so far as its relation to rust infection is concerned.

TABLE 2.—*Host reactions of certain pole beans of the Kentucky Wonder type to physiologic forms 1 and 2 of bean rust*

Variety	Form 1		Form 2	
	Characterization	Rating ¹	Characterization	Rating ¹
Basketful.....	Susceptible.....	10	Susceptible.....	10
Erntebringer (Phaenomen).....	do.....	10	do.....	10
Hildeshelmer (Phaenomen).....	Resistant.....	2	do.....	10
Kentucky Wonder (brown-seeded).....	Susceptible.....	10	do.....	10
Kentucky Wonder Wax.....	Variable.....	2-10	do.....	10
Kentucky Wonder (white-seeded).....	Susceptible.....	10	do.....	10
Meisterstuck.....	Resistant.....	2	do.....	10
Meisterstuck (selected).....	Susceptible.....	10	do.....	10
Phaenomen.....	Resistant.....	2	do.....	10
President Roosevelt.....	Susceptible.....	10	do.....	10
Schlachtschwert (original).....	Variable.....	2-10	do.....	10
Schlachtschwert (selected).....	Resistant.....	2	do.....	10
Do.....	Variable.....	2-10	do.....	10
Do.....	Susceptible.....	10	do.....	10
World Wonder.....	Resistant.....	2	do.....	10
Do.....	Susceptible.....	10	do.....	10
Zeppelin Giant.....	Resistant.....	2	do.....	9
Zeppelin Mulstopper.....	do.....	2	do.....	10

¹ Varieties graded 0 or 1 are practically immune; those graded 10 are very susceptible

An examination of table 2 shows several of these varieties to be almost entirely immune to bean rust form 1 and very susceptible to form 2. The results with World Wonder and Schlachtschwert call for special comment. The World Wonder was received in two lots; one lot shows decided resistance and the other a high degree of susceptibility to form 1, while both are very susceptible to form 2. Schlachtschwert is even more interesting, since the original lot contained seed that produced plants varying from 2 to 10 in susceptibility to form 1; no resistance was apparent to form 2. This variety has been cultivated for a few years and selections have been made from it on the basis of marketable type. Out of these selections there were obtained 1 strain resistant and 1 susceptible to form 2 and 1 that varied from 2 to 10 in susceptibility. This variety apparently is not genetically fixed when its tolerance to rust infection is considered.

Form 2 appears to be the more virulent strain and readily infects varieties that are partially to almost completely resistant to form 1. Form 1 apparently is the more widely distributed strain and greatly predominates in mixtures of the two.

After most of the studies with the two rust forms had been completed, an occasional plant of the Kentucky Wonder Wax was found to resist infection by both forms 1 and 2. The seed from the resistant plants were saved and multiplied. Additional inoculations with both strains of rust demonstrated the existence of an inherent resistance and not, as might be at first suspected, a failure to obtain infection.

No attempt has been made to exhaust the possibilities of physiologic forms. The writers feel that additional forms could probably be isolated if collections of rust were made from widely separated regions of the world and tested on a number of differential hosts. A large

number of forms have been reported for cereal rusts (3), and it is not unlikely that additional forms occur on the bean.

RUST CONTROL

In respect to the problem of prevention and control, bean rust is not unlike rusts of other crops. It is prevalent when conditions are ideal for its development, and it is difficult to control except by the planting of tolerant or resistant varieties or by the development of resistant strains by breeding and selection.

Investigations on the control of rust by the use of fungicides have been numerous. Kellerman (17), Pammel (22), Galloway (10), and others carried out spraying experiments for the purpose of controlling stem rust of oats and leaf rust of wheat, oats, and barley, but with little success. More recently several investigators, among whom may be mentioned Kightlinger (18), Bailey and Greaney (5), Lambert and Stakman (20), and Greaney (13), have attempted cereal rust control by the use of sulphur dusts. The results on the whole have been fairly consistent in demonstrating the effectiveness of sulphur in preventing rust infection. The decrease in the percentage of rust was accompanied by a corresponding increase in yield. Different methods of application were employed, and the general conclusion seems to be that the rust could be controlled if a practical method of applying the dust could be devised. The time of application with respect to infection and rains was found to be important.

GREENHOUSE EXPERIMENTS

The possible value of dusting with sulphur compounds to control bean rust, as shown by preliminary field trials, suggested the desirability of carrying out experiments in the greenhouse, where conditions such as temperature and humidity could be controlled adequately. It was also believed that the results of such trials might be useful when an opportunity was afforded to conduct additional field experiments. After inoculation the plants were confined in an infection chamber, the temperature of which was held within a range previously shown to be most favorable for infection. The humidity was held near to saturation. Commercial products known as Kolo-dust and flowers of sulphur were used, and no difference in results between the two could be observed. Young, healthy plants of the Pinto variety were used for all the dusting experiments. Some plants were dusted immediately before and some immediately after the leaves had been atomized with rust spores, and some after different intervals of time following inoculation. Perfect control was obtained whether the plants were dusted immediately before or immediately after inoculation.

These results were followed by experiments to determine how long after the plants are inoculated the dust may be applied and still be effective. Considerable interest was attached to these experiments, not alone from the standpoint of control but because it was hoped that they might give some information as to the time that elapses after the application of the rust spores before penetration takes place. A preliminary series of experiments was initiated in which a number of plants were atomized with spores and confined in an infection chamber. For a period of 5 days after inoculation

several plants were removed from the chamber every 24 hours. These plants were dusted with flowers of sulphur. The application of the sulphur had no influence whatever, the rust pustules being just as numerous on the dusted plants as on the controls. These results indicate that penetration of the host tissue had taken place in the first 24 hours. The results showed also that the plants confined in the infection chamber the longest were proportionately later in developing pustules, a condition for which the dust is not responsible but which can be attributed to the environment that was presumably unfavorable for the development of the plant and the rust.

Dusting the plants at intervals of 6, 12, and 17 hours after inoculation was next tried. Controls dusted immediately after inoculation and some not dusted were run in all the experiments. Good rust infection was obtained with the undusted controls. On the other hand, perfect control was obtained when the plants were dusted immediately after atomizing. Dusting 6 hours after inoculation gave perfect control. A few rust pustules developed on plants dusted after 12 hours, and good infection developed after a 17-hour period, though it was not quite equal to that of the undusted controls. These results show that no penetration, at least none beyond the possibility of injury by the sulphur, had taken place at the end of 6 hours. On the other hand some of the germ tubes had safely penetrated the host in 12 hours, and many of them at the end of 17 hours.

The results obtained with sulphur as a control of bean rust suggested trials with a copper-lime dust. A commercial preparation consisting of 20 parts copper sulphate and 80 parts hydrated lime was dusted on one group of plants before inoculation, and on another at intervals of 3, 6, 17, 20, 24, and 41 hours after they had been sprayed with spores (form 2). The dusted as well as the inoculated undusted controls were confined in an infection chamber for 42 hours and then removed. The results of these experiments suggested that very inadequate control, if any, could be expected from dusting with a copper-lime dust. No benefit resulted from dusting immediately before inoculation, and only a very limited reduction in infection was obtained when the plants were dusted immediately after inoculation. Similar results were obtained from repeated experiments. Judging from these results, dusting with sulphur offers more promise of control than copper-lime dust.

FIELD EXPERIMENTS

In 1928, after the 1927 epidemic of bean rust in Colorado, some field experiments were initiated in its control. There was only a limited amount of rust that year, but enough to give some idea of what might be expected should control measures be applied at the right time. The dusts used in 1928 were commercial preparations, consisting of different forms of oxidizing sulphur and one known commercially as Kolodust. There were three control plots. On July 31 there was a little rust in evidence when an application was made with a hand duster at the rate of 5 pounds per one-fourteenth of an acre. A second application was made August 7. The latter part of the season was not particularly favorable for rust development, which increased only moderately after the second dusting, so a third application was not found necessary. On September 3, final records were taken of the experiments, and it was found that 15 to

25 percent more damage had been done to the controls than to the dusted plots. Had it been a bad rust year, better results might have been obtained. The control plots yielded a fair crop. Since 1928 so little rust has occurred that no further experiments have been carried out in the field.

No general conclusions can be drawn from these experiments, but they suggest that control of rust may be possible if a practical way can be found to apply the dust. The control of bean rust by the application of liquid or dust fungicides, while possible from a seasonal standpoint, is at most only temporary and of doubtful practicability.

A number of varieties that resist the rust sufficiently for practical purposes have been tested. There is more resistance exhibited by the snap bean varieties than by the field beans. Unfortunately, the more popular and widely grown field varieties are very susceptible. Breeding and selection must be employed in order to secure substitutes for susceptible varieties that will be capable of producing a crop in rust-infected areas. In fact the evidence indicates that the control problem will never be adequately solved until resistant varieties are developed by hybridization and selection.

SUMMARY

Observations on the succession of spore forms indicate that the bean rust (*Uromyces phaseoli typica* Arthur) can be made to complete its full life cycle under greenhouse conditions.

Recognition is made of a teliospore-producing and a non-teliospore-producing strain of bean rust. Attempts to force teliospore formation reveal that the abundance of teliospores, within the teliospore-producing strain, as well as the time when they appear, may be controlled to an appreciable extent by altering host metabolism.

Teliospores 207 days old and urediospores 182 days old, were germinated. Urediospores show a decrease in germinability with age, while teliospores gave maximum germination after 6 months.

A brief comparison is made of the bean rust with the cowpea rust (*Uromyces phaseoli vignae* (Barcl.) Arthur) and rust of *Strophostyles helvola*.

The optimum temperature for spore germination in bean rust was found to be about 14.5° C., determined at the end of a 24-hour period, on a percentage basis. The optimum temperature for infection was slightly higher than that for spore germination, or approximately 17°.

No infection took place at a relative humidity below 95 percent. Infection seems to require either moisture condensation or an initial excess of moisture on the plants at the beginning of the infection period.

The presence or absence of light influenced infection. Plants sprayed with spores and confined in a moist chamber in subdued light or almost total darkness for 24 to 48 hours became normally infected. If confined for a longer time, the number of sori was somewhat reduced and their development delayed. The results point to the conclusion that an abnormality in the functioning of the host is accompanied by delayed or reduced infection.

The minimum time for infection to take place under optimum conditions is 8 hours; 12 to 18 hours constitutes about the optimum time.

Results indicate that outbreaks of bean rust are possible in any region where a relative humidity of 95 percent or more is maintained for any period of 8 hours or more.

Sixty varieties of garden and field beans (*Phaseolus vulgaris*), 15 varieties of the lima bean (*P. lunatus macrocarpus*), and the tepary bean (*P. acutifolius latifolius*) were tested for rust susceptibility and rated on a scale of 0 to 10.

A new physiologic form of bean rust was discovered and designated form 2.

Dusting with sulphur suggests an effective means of controlling bean rust.

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THE MICROBIOLOGICAL DECOMPOSITION OF THE CONSTITUENTS OF ALFALFA HAY¹

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INTRODUCTION

Investigation of the biological, chemical, and engineering aspects of the spontaneous ignition of hay and similar agricultural products has been under way in the Bureau of Chemistry and Soils for several years³ (2, 5, 6, 12, 13, 14, 15, 27, 31).⁴

It is now recognized that micro-organisms play an important role in the early stages of the decomposition which finally leads to the spontaneous ignition of plant material (7, 18). However, little detailed information can be found regarding the disappearance of the constituents of alfalfa hay when subjected to microbial decomposition either under laboratory conditions or in a heating haystack. The results of a laboratory study of this problem are presented in the present paper.

REVIEW OF LITERATURE

During recent years the literature on the microbiological decomposition of lignified plant materials has rapidly increased. Soil chemists and soil bacteriologists have been particularly active in studying this problem in the hope of gaining a better understanding of the mechanism involved in the formation of soil organic matter or humus. No attempt will be made here to review this literature as this has already been done elsewhere (8, 23, 25). It is necessary only to point out that there appears to be a decided difference, insofar as the action of fungi and bacteria is concerned, between lignin as it occurs naturally in plants and free or isolated lignin. Willstätter lignin, or lignin isolated by the 72-percent-sulphuric-acid method, appears to be resistant to the action of soil micro-organisms and to the wood-destroying fungi *Trametes pini* and *Polystictus hirsutus* (11, 30, 35). Pringsheim and Fuchs (26), and more recently Denme (8), have shown that lignin isolated by the alkali method can be demethoxylated by soil micro-organisms. Under aerobic conditions natural lignin can be decomposed by soil micro-organisms, at least in part, although the rate of decomposition is generally less than that of cellulose and hemicelluloses (3, 25, 34). Recently Boruff and Buswell (4) reported that they were unable to ferment isolated lignin under anaerobic conditions, though the natural lignin in cornstalks could be fermented under these conditions. This is in opposition to the previous findings of Tenney and Waksman (32, 33), who

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³ U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF CHEMISTRY AND SOILS. REPORT OF CONFERENCE ON SPONTANEOUS HEATING AND IGNITION OF AGRICULTURAL AND INDUSTRIAL PRODUCTS, CALLED BY NATIONAL FIRE PROTECTIVE ASSOCIATION, IN COOPERATION WITH U. S. DEPARTMENT OF AGRICULTURE AND U. S. DEPARTMENT OF COMMERCE, WASHINGTON, D. C., NOV. 14 AND 15, 1929. 137 pp. [Multigraphed.]

⁴ Reference is made by number (italic) to Literature Cited, p. 773.

reported that under anaerobic conditions the natural lignin of alfalfa was preserved practically quantitatively. In a recent article Juon (16) reported that green fodders subjected to fermentation suffered a loss of lignin. In most cases, however, the loss was rather small. In general, it was found that lignin was more resistant to the action of micro-organisms than the other major plant constituents.

EXPERIMENTAL PROCEDURE

In studying the microbiological decomposition of alfalfa hay in the laboratory, two types were selected, one representing the first crop of the season (referred to in this paper as "first cutting"), and the other representing the third crop ("third cutting"). The samples of each cutting were collected fresh from the field, sun-dried for several days, then ground fine in a mill. For analysis a subsample was dried in an oven at 105° C. The following determinations were made. The percentage of ash was calculated on the basis of oven-dried material, and all the other results were calculated on the basis of oven-dried, ash-free material.

Ash.—This was determined in the usual manner by igniting a weighed sample with a bunsen burner and weighing the inorganic residue.

Alcohol-benzene extractives.—These were determined by extracting a weighed sample in a Soxhlet extractor for 30 hours with a 1:2 alcohol-benzene solution and ascertaining the loss in weight.

Cold-water extractives.—To a weighed sample of the dry material which had been extracted with the alcohol-benzene solution, distilled water was added in the proportion of 150 cc to 1 g of sample and allowed to digest at room temperature, with frequent stirring, for 48 hours. The loss in weight after extracting and redrying was calculated on the basis of the original unextracted ash-free material.⁵

Hot-water extractives.—A weighed sample of the dry residue from the previous extraction was treated with distilled water (in the proportion of 150 cc of water to 1 g of sample) and boiled under the reflux condenser for 3 hours. The loss in weight was calculated on the basis of the original dry unextracted ash-free material.⁵

Uronic acid anhydrides.—These were determined in the dry unextracted material according to the procedure recommended by Dickson, Otterson, and Link (9) as modified slightly by Phillips, Goss, and Browne (24). The percentage of uronic acid anhydrides in a plant material is a measure of its total polyuronide content. They may be present in pectins or in hemicelluloses.

Pentosans.—The percentage of furfural in the dry unextracted sample was determined by the method of the Association of Official Agricultural Chemists (1). One-sixth of the percentage of uronic acid anhydrides was deducted from this, and the result was calculated as percentage pentosans.

Cross and Bevan cellulose.—This was determined by the method of Norman and Jenkins (20), but instead of the filter suggested by these authors, Jena sintered glass crucibles were employed.

Furfural in Cross and Bevan cellulose.—This was determined by the method of the Association of Official Agricultural Chemists (1). The phloroglucide precipitate was washed with 100 cc of ethanol

⁵ In view of the fact that the water-soluble ash was rather small, no attempt was made to determine the percentage of ash in the extracts.

(heated to 60° C.) to remove any phloroglucide of methyl-furfural present.

Cellulose.—The Cross and Bevan cellulose consists essentially of two components, a true cellulose fraction and a furfural-yielding fraction, which in all probability is xylan (19). In order to ascertain the true cellulose content of the material, the percentage of furfural in the Cross and Bevan cellulose was determined, as already indicated, and the result calculated as percentage pentosans. The result thus obtained when deducted from the percentage of Cross and Bevan cellulose gave the percentage cellulose in the sample. Thus, if C equals the percentage of Cross and Bevan cellulose, P the percentage of pentosans in the Cross and Bevan cellulose, the percentage of cellulose equals $C - (C \times P/100)$.

Lignin.—The weighed sample which had been successively extracted with a 1:2 alcohol-benzene solution, cold water, and hot water, according to the procedures described above, was treated with a 1-percent hydrochloric acid solution, in the proportion of 1 g of plant material to 150 cc of acid, and boiled under the reflux condenser for 3 hours. The insoluble residue was filtered off, and dried at 105° C., and the loss in weight was determined. The extracted material was analyzed for lignin by the fuming hydrochloric acid method according to the procedure described by Phillips (22). Three samples were weighed out, and in the lignin residues from the first two samples the percentages of ash and nitrogen were determined, and in the lignin fraction from the third sample the percentage of methoxyl was determined. Corrections were made for the nitrogen and ash in the lignin.

Methoxyl in ash-free lignin.—The lignin residue obtained from the third sample used in the determination of lignin was collected in a Pyrex sintered glass crucible (2 cm inside diameter and 5 cm high). The weight of lignin, corrected for ash and crude protein, was determined. The percentage of methoxyl in this was determined by the procedure described by Phillips (22). The methyl iodide was absorbed in pyridine as recommended by Kirpal and Bühn (17). A slightly modified Zeisel apparatus (22) was used. The reaction flask consisted of the outer cone of a no. 30 interchangeable ground glass joint, the unground end of which had been sealed off and rounded out. By means of this arrangement it was possible to insert the glass crucible containing the lignin into the reaction flask. To the condenser of the Zeisel apparatus, the inner cone of a no. 30 interchangeable ground glass joint was sealed.

Methoxyl in unextracted plant material.—The percentage of methoxyl in the dried unextracted plant material was determined as described above. The methyl iodide was absorbed in pyridine.

Methoxyl in extracted plant material.—The method generally used for the determination of the percentage of methoxyl in an organic substance is based on a reaction which all ethers undergo when heated with hydriodic acid. The reaction may be represented as follows: $ROR' + HI = ROH + R'I$, in which R and R' may be either alkyl or aryl groups. If R' is a methyl group, that is, if the substance contains a methoxyl group, then methyl iodide is formed as a result of the reaction, and this may be absorbed either in pyridine or in an alcoholic silver nitrate solution and quantitatively estimated. However, if methyl esters are also present, these two react with hydriodic

acid and yield methyl iodide. Accordingly, when a plant material is heated with hydriodic acid not only do the methoxyl groups of methyl ethers react but also those in esterlike combination, as in the case of pectins and in methyl esters of organic acids generally. If the plant material is subjected to a preliminary hydrolysis with dilute acid, however, the methoxyl groups attached in etherlike combination are very little affected by this treatment, whereas the methyl groups of methyl esters are split off. It is now known definitely that the methoxyl groups in lignin are attached in etherlike combination, so that by determining the percentage of methoxyl in a lignified plant material, such as alfalfa which has been subjected to a preliminary hydrolysis with dilute acid, the percentage of methoxyl obtained represents essentially, although perhaps not entirely, lignin methoxyls. It will be observed that the weight of methoxyl in the extracted plant material is in every case greater than the actual quantity of methoxyl in the lignin (weight of ash-free lignin \times percentage of methoxyl in the lignin). This difference is small, however, and may be due either to (1) a partial demethoxylation of the lignin brought about in the course of its isolation from the plant material, or (2) to firmly bound methoxyl groups occurring in some component of alfalfa other than lignin. It has been shown by O'Dwyer (21), Schmidt and his coworkers (29), and by Hägglund and Sandelin (10) that in wood some firmly bound methoxyl groups are also in the carbohydrate fraction. The determination of the percentage methoxyl in a lignified plant material that had been treated in the manner described above, however, does give an approximate idea of the quantity of lignin present. This determination was carried out as follows: The weighed sample was successively extracted with a 1:2 alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution under conditions previously described, and the loss in weight was determined. The percentage of methoxyl in the extracted sample was determined by the method already described in the paragraph headed "Methoxyl in plant material", and the result calculated on the basis of the original unextracted ash-free material.

Crude protein ($N \times 6.25$).—This was determined in the unextracted plant material in the usual manner by the Kjeldahl, Gunning, Arnold method (1).

The terms "pentosans", "lignin", and "cellulose" as well as those used for the other constituents in this paper refer only to plant substances or plant fractions as defined by the analytical methods employed.

In all the experiments both first-cutting and third-cutting alfalfa were used. In every experiment sun-dried ground hay was employed (equivalent to 20 g oven-dried (105° C.) material). The several samples were placed in Erlenmeyer flasks, and to each a sufficient quantity of a modified Czapek's solution was added as the nutrient medium. In place of the sodium nitrate in the regular Czapek solution an equivalent quantity of urea was substituted, and the sugar generally used was omitted. In a previous investigation by Phillips, Weihe, and Smith (25) it was found that when this modified Czapek's solution was used the reaction of the medium remained almost neutral throughout the experiment. In all experiments conducted under aerobic conditions the pH of the medium after fermentation ranged from 7.0 to 8.0. In the anaerobic experiments the pH of the medium

after fermentation ranged from 5.0 to 6.0. As the fermentation of hay by the natural microflora was to be studied, no inoculations were made. Although no microbial analyses of the fermented hay were made, it was apparent that most of the samples had undergone decomposition by bacteria. A few of the samples showed evidence of decomposition by actinomycetes, and since their reactions were distinctly acid, the fermented plant materials were not subjected to chemical analyses.

In the first series of experiments two sets of cotton-stoppered flasks, one containing hay of the first cutting and the other hay of the third cutting, each with nutrient medium, were incubated for 30 days at 30° C. In this series each experiment was conducted in duplicate. In the second series the flasks containing the ground hay and nutrient medium were incubated first for 30 days at 30° and then for 30 days at 55°. In this series each experiment was conducted in triplicate. In the third series the flasks containing the ground hay and nutrient medium were each closed with a rubber stopper containing a mercury seal and were incubated under anaerobic conditions for 30 days at 30°. In this series the duplicate samples were combined and thoroughly mixed, and the composite sample was analyzed.

At the end of each incubation period in the first three series of experiments the residual plant material in each flask was filtered off, dried at 105° C., and weighed. From the weight of the material in each flask before and after fermentation the percentage loss due to microbial action was calculated. The composition of the residual plant material was then determined by the analytical methods previously described. By comparing these results with those obtained in the analysis of the materials in the original state, data were obtained showing the absolute loss of each constituent or group of constituents brought about by the action of the micro-organisms. The composition of the starting material is given in table 1. The results of the first, second, and third series of experiments are recorded in tables 2, 3, and 4, respectively.

TABLE 1.—Composition of alfalfa used in microbiological decomposition experiments¹

[Percentage of ash was calculated on the basis of oven-dried material. All other results were calculated on the basis of oven-dried, ash-free material]

Constituents	First cutting		Third cutting	
	Percent	Grams	Percent	Grams
Ash.....	5.80		5.97	
Alcohol-benzene extractives.....	23.7	4.4650	27.3	5.1324
Cold-water extractives.....	10.8	2.0347	12.8	2.4064
Hot-water extractives.....	5.3	.9985	6.0	1.1280
Uronic acid anhydrides.....	11.6	2.1854	12.0	2.2560
Pentosans ²	15.2	2.8636	11.6	2.1808
Cross and Bevan cellulose.....	30.9	5.8215	23.2	4.3614
Furfural in Cross and Bevan cellulose.....	13.8		12.5	
Cellulose.....	23.6	4.4462	18.2	3.4216
Lignin.....	9.7	1.8274	7.6	1.4288
Methoxyl in ash-free lignin.....	11.8		9.7	
Methoxyl in plant material.....	2.8	.5275	2.6	.4888
Methoxyl in extracted plant material.....	1.5	.2826	1.0	.1890
Crude protein. N×6.25.....	16.2	3.0520	21.3	4.0044

¹ 20 grams of dry material was used for each experiment.

² Corrected for furfural from uronic acids.

In the fourth series of experiments the course of the fermentation was followed by measuring the daily evolution of carbon dioxide from flasks containing hay and nutrients, and fermented under conditions similar to those of the second series—that is, for 30 days

at 30° C. and then for 30 days at 55°. Duplicate samples of hay from each cutting were prepared as described above, and the flasks were connected to the carbon dioxide absorbing apparatus described by Beavens and James (2). Daily titrations of the carbon dioxide evolved from the fermenting hay were made, and the results were recorded as milligrams of carbon.

RESULTS

For the sake of convenience, the results presented in each table will be dealt with separately. Table 1 shows that there was a decided difference in composition in the two cuttings of hay. This difference was reflected in the behavior of the two materials toward the action of micro-organisms as measured by the percentage loss of the various constituents and by the quantity of carbon dioxide evolved. Table 1 also shows that although the percentage of ash in the two cuttings of hay was about the same, the percentage of the other constituents was substantially different. In every case of the alcohol-benzene, cold and hot water extractives, uronic acid anhydrides, and crude protein ($N \times 6.25$) a greater percentage was found in the third-cutting than in the first-cutting alfalfa. However, in the pentosans, Cross and Bevan cellulose, furfural in Cross and Bevan cellulose, cellulose, lignin, methoxyl in lignin, and methoxyl in extracted and unextracted plant materials, the relationship was reversed. The first-cutting alfalfa showed greater percentages of these constituents than the third-cutting alfalfa.

TABLE 2.—Composition of alfalfa fermented for 30 days at 30° C. under aerobic conditions ¹

FIRST CUTTING ¹

[All results were calculated on the basis of oven-dried, ash-free material]

Constituents	Sample 1			Sample 2		
	In alfalfa		Loss ²	In alfalfa		Loss ²
	Percent	Grams	Percent	Percent	Grams	Percent
Alcohol-benzene extractives.....	9.2	0.9236	79.3	9.1	0.8508	80.9
Cold-water extractives.....	10.3	1.0341	49.1	11.5	1.0752	47.1
Hot-water extractives.....	3.7	.3714	62.8	3.4	.3179	48.1
Uronic acid anhydrides.....	7.4	.7429	66.0	7.0	.6545	68.1
Pentosans ³	15.5	1.5562	45.6	16.0	1.4960	65.4
Cross and Bevan cellulose.....	43.0	4.3172	25.8	45.1	4.2168	70.0
Furfural in Cross and Bevan cellulose.....	12.4	12.4	68.0
Cellulose.....	33.9	3.4035	23.4	35.5	3.3192	47.7
Lignin.....	17.0	1.7068	6.6	18.3	1.7110	46.6
Methoxyl in ash-free lignin.....	14.1	13.9	27.5
Methoxyl in plant material.....	3.4	.3413	35.3	4.1	.3833	28.6
Methoxyl in extracted plant material.....	2.8	.2811	.5	3.0	.2805	6.3
Crude protein, $N \times 6.25$	12.8	1.2851	57.8	14.0	1.3090	6.4

¹ 20 grams of dry material was used for each experiment. This corresponded to 18.84 and 18.80 g of ash-free material from the first and third cuttings, respectively.

² The weight of the dry, ash-free, fermented hay from the 2 samples at the end of the experiment was 10.04 and 9.35 g, respectively, which was 46.7 and 50.3 percent loss in weight, respectively.

³ Calculated from data in table 1.

⁴ The mean value of the 2 samples.

⁵ Corrected for furfural from uronic acids.

TABLE 2.—Composition of alfalfa fermented for 30 days at 30° C. under aerobic conditions—Continued

THIRD CUTTING ⁴

Constituents	Sample 1			Sample 2		
	In alfalfa		Loss ³	In alfalfa		Loss ³
	Percent	Grams		Percent	Grams	Percent
Alcohol-benzene extractives	14.1	1.2831	75.0	13.5	1.2366	75.9
Cold-water extractives	9.5	.8645	64.0	11.6	1.0625	⁴ 75.4 55.8
Hot-water extractives	3.5	.3185	71.7	4.1	.3755	⁴ 59.9 66.7
Uronic acid anhydrides	6.8	.6188	72.5	6.9	.6320	⁴ 69.2 71.9
Pentosans ⁵	12.6	1.1466	47.4	12.5	1.1450	⁴ 72.2 47.4
Cross and Bevan cellulose	33.2	3.0212	30.7	33.2	3.0411	⁴ 47.4 30.2
Furfural in Cross and Bevan cellulose ..	12.5			12.3 ⁴ 12.4		⁴ 30.4
Cellulose	26.1	2.3751	30.5	26.2	2.3999	29.8
Lignin	14.9	1.3559	5.1	14.5	1.3282	⁴ 30.1 7.0
Methoxyl in ash-free lignin	11.2			10.6 ⁴ 10.9		⁴ 6.0
Methoxyl in plant material	3.0	.2730	44.1	2.8	.2564	47.5
Methoxyl in extracted plant material ..	2.0	.1820	3.2	1.9	.1740	⁴ 45.8 7.4
Crude protein, N×6.25	18.6	1.6926	57.7	19.0	1.7404	⁴ 5.3 56.5 ⁴ 57.1

¹ Calculated from data in table 1² The mean value of the 2 samples.³ Corrected for furfural from uronic acids⁴ The weight of the dry, ash-free, fermented hay from the 2 samples at the end of the experiment was 9.10 and 9.16 g, respectively, which was 51.6 and 51.2 percent loss in weight, respectively.

The results of microbial decomposition of alfalfa hay under aerobic conditions when allowed to proceed for 30 days at 30° C. is shown in table 2. It will be observed that the total decomposition as measured by the percentage loss in weight was slightly less in the first-cutting alfalfa than in the third-cutting alfalfa. The alcohol-benzene extractives showed a somewhat greater loss in the first-cutting than in the third-cutting material. However, in the cold- and hot-water extractives and uronic acid anhydrides this relationship was reversed, although the differences were not large. The percentage loss of pentosans and crude protein (N×6.25) in the two plant materials was about the same. The Cross and Bevan cellulose, cellulose, and methoxyl in the plant material (unextracted) showed a greater loss in the third-cutting than in the first-cutting material. The percentage of furfural in the Cross and Bevan cellulose of the first-cutting material decreased somewhat, although in the third-cutting material no such effect was noted. The loss of lignin in the plant materials of both cuttings was of the same general order of magnitude. In the third-cutting material the loss of lignin is indicated not only in the results obtained by direct analysis for lignin but also by the loss of methoxyl in the extracted plant material. There is no explanation for the discrepancy between the loss of lignin in the first-cutting material and the small loss in the methoxyl in extracted plant material. In the main, the results indicate that under the conditions of this series of experiments the lignin fraction was the most resistant to microbial attack, although it did suffer some decomposition.

TABLE 3.—Composition of alfalfa fermented for 30 days at 30° C. and for 30 days at 55° C. under aerobic conditions ¹

[All results calculated on the basis of oven-dried, ash-free material]

FIRST CUTTING ²

Constituents	Sample 1			Sample 2			Sample 3		
	In alfalfa		Loss ³	In alfalfa		Loss ³	In alfalfa		Loss ³
	Percent	Grams	Percent	Percent	Grams	Percent	Percent	Grams	Percent
Alcohol-benzene extractives	15.6	1.2292	72.4	13.4	1.2381	72.2	14.9	1.3007	70.8
Cold-water extractives.....	8.0	.6304	69.0	7.0	.6468	68.2	8.7	.7595	71.8
Hot-water extractives.....	3.1	.2442	75.5	3.2	.2956	70.4	3.4	.2968	62.6
Uronic acid anhydrides.....	6.8	.5358	75.4	6.1	.5636	74.2	6.2	.5412	66.6
Pentosans ⁴	16.4	1.2923	54.8	15.7	1.4506	49.3	15.0	1.3095	70.2
Cross and Bevan cellulose..	36.8	2.8998	50.1	43.4	4.0101	31.1	38.3	3.3435	72.0
Furfural in Cross and Bevan cellulose.....	13.5			13.0			13.4		75.2
Cellulose.....	28.3	2.2300	49.8	33.8	3.1231	29.7	29.5	2.5753	74.9
Lignin.....	19.2	1.5129	17.2	19.1	1.7648	3.4	18.7	1.6325	54.2
Methoxyl in ash-free lignin.	11.3			12.0			10.7		52.7
Methoxyl in plant material.	3.4	.2679	49.2	4.2	.3880	26.4	4.0	.3492	42.5
Methoxyl in extracted plant material.....	2.6	.2048	27.5	2.7	.2494	11.7	2.6	.2269	41.2
Crude protein, N×6.25.....	13.7	1.0795	64.6	10.8	.9979	67.3	12.2	1.0650	

THIRD CUTTING ⁵

Constituents	Sample 1			Sample 2			Sample 3		
	In alfalfa		Loss ³	In alfalfa		Loss ³	In alfalfa		Loss ³
	Percent	Grams	Percent	Percent	Grams	Percent	Percent	Grams	Percent
Alcohol-benzene extractives.	20.4	1.8686	63.5	23.0	1.6744	67.3	17.2	1.4396	71.9
Cold-water extractives.....	10.5	.9618	60.0	11.8	.8590	64.3	7.3	.6110	67.6
Hot-water extractives.....	2.8	.2564	77.2	3.8	.2706	75.4	2.5	.2092	74.6
Uronic acid anhydrides.....	6.7	.6137	72.7	7.3	.5314	76.4	5.6	.4687	69.3
Pentosans ⁴	12.0	1.0992	49.6	12.3	.8954	58.9	12.0	1.0044	81.4
Cross and Bevan cellulose.	34.8	3.1876	26.9	36.0	2.6208	39.9	32.6	2.7286	78.0
Furfural in Cross and Bevan cellulose.....	12.4			12.5			12.4		79.2
Cellulose.....	27.4	2.5098	26.6	28.3	2.0602	39.7	25.7	2.1510	76.1
Lignin.....	14.7	1.3465	5.7	15.0	1.0920	23.5	13.5	1.1299	53.9
Methoxyl in ash-free lignin.	9.1			9.9			10.1		54.1
Methoxyl in plant material.	2.7	.2473	49.4	2.7	.1965	50.8	2.4	.2008	37.4
Methoxyl in extracted plant material.....	2.0	.1832	2.5	2.0	.1456	22.5	1.9	.1590	34.7
Crude protein, N×6.25.....	13.9	1.2732	68.2	19.8	1.4414	64.0	12.8	1.0713	

¹ 20 g of dry material was used for each experiment. This corresponded to 18.84 and 18.90 g of ash-free material from the first and third cuttings, respectively.² The weight of dry, ash-free, fermented hay from the 3 samples at the end of the experiment was 7.88, 9.26, and 8.73 g, respectively, which was 59.1, 50.9, and 53.6 percent loss in weight, respectively.³ Calculated from data in table 1.⁴ The mean value of the 3 samples.⁵ Corrected for furfural from uronic acids.⁶ The weight of the dry, ash-free, fermented hay from the 3 samples at the end of the experiment was 9.16, 7.23, and 8.37 g, respectively, which was 51.2, 61.2, and 55.5 percent loss in weight, respectively.

In table 3 figures are presented showing the composition and percentage loss suffered by the several constituents of alfalfa hay when fermented under aerobic conditions, first for 30 days at 30° C. and then for an additional 30 days at 55°. In this series of experiments, the results indicate the combined effect of the decomposition by mesophilic and thermophilic micro-organisms and the longer incubation period. It will be observed that, as in the previous series of experiments (table 2), the total decomposition as measured by the average percentage loss of the organic fraction of the plant materials was slightly greater in the third-cutting than in the first-cutting alfalfa. The alcohol-benzene extractives showed a greater loss in the first-cutting than in the third-cutting material, as was also the case in the previous series of experiments (table 2). The average loss of cold-water extractives was about the same in both cuttings. It was, however, considerably greater than when the plant materials were fermented for 30 days at 30° (table 2). In general, this was the case with all the other constituents except the alcohol-benzene extractives. The hot-water extractives, uronic acid anhydrides, pentosans, methoxyl in plant material (unextracted), and crude protein ($N \times 6.25$) suffered a somewhat greater loss in the third-cutting than in the first-cutting material. In the Cross and Bevan cellulose and the cellulose proper, the reverse was true; that is, the first-cutting material suffered the greater percentage loss. The percentage of furfural in the Cross and Bevan cellulose remained substantially the same as in the original plant materials, thus indicating that the micro-organisms did not preferentially attack the xylan fraction of the Cross and Bevan cellulose. Apparently the "pure" cellulose fraction and the xylan fraction of the Cross and Bevan cellulose were attacked at approximately the same rate. The principal difference between this series of experiments and the previous one lies in the results obtained on the microbiological decomposition of lignin. In the main, the lignin from both the first and third cuttings of alfalfa suffered a greater loss than in the previous series of experiments (table 2). The results are rather irregular, but this was evidently not caused by any error in the analytical procedure, as the results on the loss of methoxyl in the extracted plant material show a similar tendency. In working with mixed cultures of organisms it is, of course, difficult to make experimental conditions identical. Under optimum conditions, the organisms capable of breaking down lignin themselves or in symbiosis with other organisms become more active, and there is accordingly a correspondingly greater loss of lignin than when conditions are not most suitable for their activity. The percentage of methoxyl in the residual lignin of this series was about the same as in the lignin from the original materials.

TABLE 4.—Composition of alfalfa fermented for 30 days at 30° C. under anaerobic conditions ¹

[All results calculated on the basis of oven-dried ash-free material]

Constituents	First cutting ²			Third cutting ²		
	In alfalfa		Loss ³	In alfalfa		Loss ³
	Percent	Grams		Percent	Grams	Percent
Alcohol-benzene extractives.....	9.0	1.0197	77.1	11.6	1.0718	79.1
Cold-water extractives.....	4.9	.5551	72.7	5.3	.4897	79.6
Hot-water extractives.....	3.6	.4078	59.1	3.7	.3418	69.7
Uronic acid anhydrides.....	8.4	.9517	56.4	8.2	.7576	66.4
Pentosans ⁴	17.3	1.9600	31.5	15.2	1.4044	35.6
Cross and Bevan cellulose.....	46.4	5.2571	9.7	40.8	3.7699	13.5
Furfural in Cross and Bevan cellulose.....	12.6			12.0		
Cellulose.....	36.4	4.1241	7.2	32.4	2.9973	12.4
Lignin.....	14.5	1.6428	10.1	14.2	1.3120	8.1
Methoxyl in ash-free lignin.....	13.4			10.0		
Methoxyl in plant material.....	3.1	.3512	33.4	2.5	.2310	52.7
Methoxyl in extracted plant material.....	2.2	.2492	11.8	1.9	.1755	6.6
Crude protein, N×6.25.....	14.2	1.6088	47.2	17.2	1.5892	60.3

¹ 20 grams of dry material was used for each experiment. This corresponded to 18.84 and 18.80 g of ash-free material from the first and third cuttings, respectively.

² The weight of dry, ash-free, fermented hay from the first and third cuttings was 11.33 and 9.24 g, respectively, which was 39.8 and 50.8 percent loss in weight, respectively.

³ Calculated from data in table 1.

⁴ Corrected for furfural from uronic acids.

In table 4 results are presented showing the effect when first and third cuttings of alfalfa are fermented for 30 days at 30° C. under anaerobic conditions. In this series of experiments also there was a greater loss in weight in the third-cutting material than in that from the first cutting. The alcohol-benzene extractives, cold- and hot-water extractives, uronic acid anhydrides, pentosans, Cross and Bevan cellulose, cellulose, methoxyl in plant material (unextracted), and crude protein (N×6.25) suffered a greater loss in the third-cutting than in the first-cutting material. The furfural-yielding fraction of the Cross and Bevan cellulose was, in part, decomposed. The Cross and Bevan cellulose fraction, it will be noted, decomposed less under anaerobic conditions than under aerobic conditions. What came as a surprise, however, was that the lignin in the alfalfa from both cuttings was decomposed as greatly under anaerobic conditions as under aerobic conditions. It is generally believed that lignin is rather resistant to microbial attack under anaerobic conditions. The results, however, are in agreement with the recent findings of Boruff and Buswell (4), who reported a loss of lignin in corncobs that had been fermented anaerobically. The results on the loss of methoxyl in the extracted plant material in the main support the results on the loss of lignin obtained by direct analysis for lignin. As compared with the other constituents, with the possible exception of the cellulose, the lignin proved to be the most resistant to microbial attack. As in table 2, the methoxyl in the residual lignin was also somewhat greater than in the lignin from the original plant materials.

The results of the fourth series of experiments are shown graphically in figures 1 and 2. The samples were incubated for 30 days at 30° C. and then for an additional 30 days at 55°. The total quantities of carbon dioxide evolved (plotted as milligrams of carbon) during the aerobic decomposition are shown in figure 1. During both incubation periods (first period, 30 days at 30°; second period,

30 days at 55°) a greater loss of carbon (as carbon dioxide) occurred in the third-cutting material than in the first-cutting material. During the first few days of the first incubation period, the difference in the quantities of carbon dioxide evolved from both the first- and third-cutting hay was greater than in the remainder of the test period. Although in the second incubation period the third-cutting material showed a more active decomposition than the first-cutting material, it is apparent from figure 1 that the two curves have a tendency to converge near the end of the experimental period.

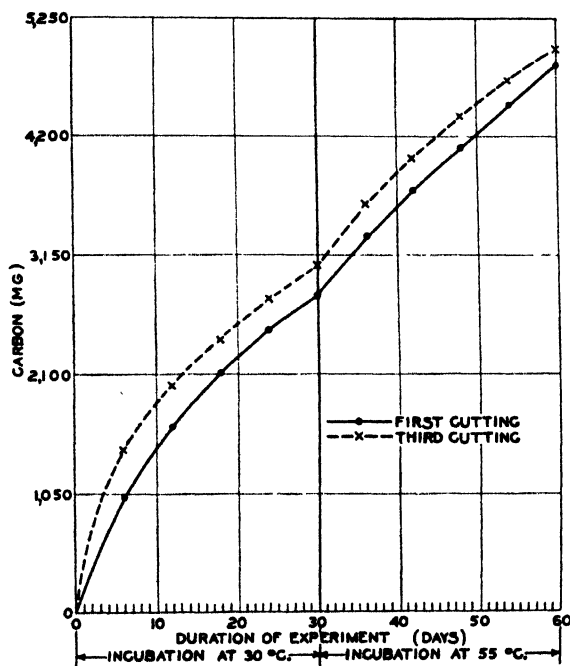


FIGURE 1.—Total carbon dioxide (calculated as carbon) evolved during the microbial decomposition of two cuttings of alfalfa hay under aerobic conditions. (Twenty grams of hay was used in each experiment.)

The results of the daily titrations of carbon dioxide evolved (plotted as milligrams of carbon) during the aerobic decomposition of the two cuttings of alfalfa hay are shown graphically in figure 2. It will be observed that the greatest rate of decomposition occurred during the first 4 to 5 days of the first incubation period (30 days at 30° C.), after which it gradually decreased. During the second incubation period (30 days at 55°) another definite increase occurred in the decomposition of both cuttings of hay, although it was not so great as in the first period of incubation. This was evidently caused by a change from the mesophilic type of microflora to the thermophilic type and a speeding up of their metabolic activities, resulting in increased decomposition. Consideration has been given to the fact that an increase in temperature of a carbon dioxide solution from 30° to 55° will, of course, cause some liberation of this gas, although one would expect this to occur shortly after the rise in temperature.

However, as is shown in figure 2, the maximum quantity of carbon dioxide was not evolved until the third day of the second period of fermentation, so that, in the main, the increased evolution of carbon dioxide may be considered as caused by increased microbial activity.

DISCUSSION

Investigators of spontaneous ignition of hay and similar agricultural materials now recognize that micro-organisms play an important role in causing the initial rise in temperature of the fermenting plant material (7, 18). However, the rise in temperature from about 70° to 80° C., when all microbial activity has ceased, to the ignition temperature of the plant material has been the subject of much speculation. No attempt will be made here to review

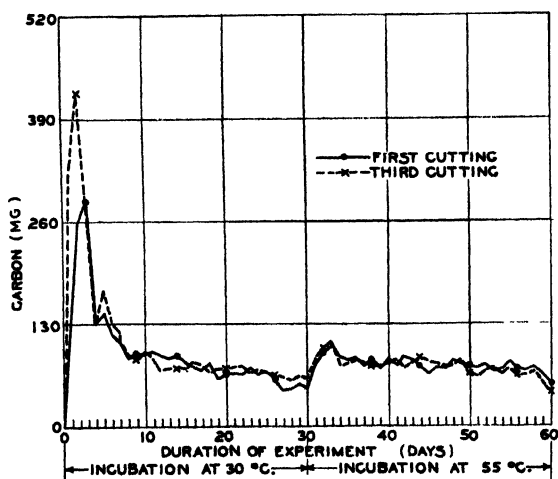


FIGURE 2. --Carbon dioxide (calculated as carbon) evolved daily during the microbial decomposition of two cuttings of alfalfa hay. (Twenty grams of hay was used in each experiment)

the various hypotheses proposed to account for this phenomenon,⁶ as this has already been done by Browne (5, 6), who put forward the hypothesis that the rise in temperature is brought about by the oxidation of unsaturated compounds produced as a result of the dehydroxylation of the carbohydrates by micro-organisms. While the oxidation of such unsaturated degradation products from carbohydrates may account for the rise in temperature of the vegetable material, attention is called to another class of unsaturated compounds, namely, the lignins, that may play an important role in bringing about the spontaneous ignition of plant materials. From tables 2, 3, and 4 it will be observed that because of the more rapid decomposition of the other major plant constituents, such as the carbohydrates, fats, proteins, and polyuronides, there is a large proportion of lignin in the residual plant material. Isolated lignin, that is, lignin prepared in the laboratory, is partly unsaturated, as

⁶ After the present paper had been prepared for publication an article by Rudge (28) appeared, suggesting that spontaneous combustion may be caused entirely by chemical action. He assumes that cellulose reacts with bicarbonates to form a condensation product having a composition analogous to that of cellulose xanthate. It is suggested that the cellulose-bicarbonate condensation product may undergo rapid oxidation, resulting under favorable circumstances in the attainment of the ignition point.

is evident from the fact that it will absorb iodine from an iodine solution. It is conceivable that lignin freed from its combination with the carbohydrates by microbial activity may be even more unsaturated, as it is reasonable to suppose that lignin liberated under comparatively mild conditions would be less polymerized. Under suitable conditions this free lignin may readily absorb oxygen, with the evolution of heat. This rise in temperature will cause a degradation of the lignin such as is produced when this substance is subjected to dry distillation. Under these conditions lignin produces, in addition to other substances, a whole series of polyhydroxy phenols (23). These phenols are related to catechol and pyrogallol, which under suitable conditions absorb oxygen with avidity. This will bring about a further rise in temperature until the ignition point of the plant material is reached.

SUMMARY

A study was made of the microbiological decomposition of first- and third-cutting alfalfa hay under aerobic conditions at incubation temperatures of 30° and 55° C. and under anaerobic conditions at 30°. The third-cutting material decomposed somewhat more rapidly than the first-cutting material. The greatest decomposition took place in the alcohol-benzene extractives, the hot- and cold-water extractives, the uronic acid anhydrides (the pectins and the polyuronide fraction generally), the crude protein ($N \times 6.25$), and the methoxyl in the plant material (unextracted). The pentosans decomposed somewhat more slowly, and the rate of decomposition of the Cross and Bevan cellulose and of the "pure" cellulose was still less. In general, the lignin was the most resistant of all the major plant constituents, although substantial losses of lignin occurred under aerobic and anaerobic conditions. The possible role of lignin in the spontaneous ignition of hay is discussed.

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SOME FACTORS INFLUENCING THE YIELD AND MORTALITY OF PONDEROSA PINE IN THE SOUTHWEST¹

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INTRODUCTION

A knowledge of those factors influencing the yield of timber stands that are more or less subject to control by man is of practical importance to the forester. The following analysis of the effect on yield of the factors of volume left after cutting and the average size of reserved trees was undertaken in an effort to add to that knowledge. Data for such a study were available from permanent sample plots established during the past 20 years by the Southwestern Forest and Range Experiment Station on cut-over areas in the ponderosa pine type for the purpose of determining the possible yield of saw timber at the end of a cutting cycle. Analyses of these plots were made by Krauch in 1926² with particular emphasis on the individual tree. In the present study, unit of area (1 acre) has been substituted as the basis of analysis, a method that lends itself readily to statistical treatment.

Because analyses of the relation of increment to time based on periods of 20 years and less usually lead to dangerous extrapolation and are of little practical assistance in predicting yield, this relation was not considered except in a preliminary fashion, and the analysis was centered on the yield at the end of 15 years, in which sense time could be considered as held constant.

MATERIAL AND METHODS

Ninety-three 1-acre plots on which growth records had been taken over a period of at least 15 years were used as the basis of the study. All plots were established immediately after logging, and hence represented the total change subsequent to cutting. There is a rather wide diversity of soil conditions among the plots, but no claim is made of exact representativeness of the universe of cut-over ponderosa pine (*Pinus ponderosa* Lawson).

For each 1-acre plot the following values were determined: (1) Volume of reserved stand; (2) volume of the average tree; (3) increment at the end of 15 years; and (4) total mortality by 5-year periods. All quantities are given in Scribner board feet, the merchantability limits being 10 inches d. b. h.³ and an 8-inch top diameter.

The reserved volume on each plot was determined by the use of volume tables. In order to eliminate so far as possible any biased

¹ Received for publication Jan. 29, 1935; issued July 1935.

² KRAUCH, H. THE DETERMINATION OF INCREMENT IN CUT-OVER STANDS OF WESTERN YELLOW PINE IN ARIZONA. Jour. Agr. Research 32: 501-541, illus. 1926.

THE DETERMINATION OF INCREMENT IN CUT-OVER STANDS OF WESTERN YELLOW PINE IN THE SOUTHWEST. Jour. Forestry 28: 978-986, illus. 1930.

³ Diameter breast high, or $4\frac{1}{2}$ feet above the ground.

errors and to keep the random errors as small as possible, separate volume tables were used for those plots representing two widely different soil types. Separate tables were used also for the two broad age classes, yellow pine and blackjack.⁴ Trees that died during the period after cutting were removed from the reserved volume in order to prevent excessive variation in yield and are treated separately under Mortality Analysis (p. 783).

The volume of the average tree on each acre was determined by dividing the total reserved volume by the number of merchantable trees. This variable was selected because it gave some indication of the size, and roughly of the age, of the reserved stand left on each plot.

The increment was found by simply subtracting the original volume of the reserved stand from the volume at the end of 15 years. No allowance was made for a possible change in form of the reserved trees after cutting, although such a change creates a small biased error in the increment values.

In the following analysis advantage was taken of correlation methods and of some of the analytical systems developed by Fisher.⁵ The influence of random errors in the independent and dependent variables on a few of the more important statistical constants was kept in mind. It has been demonstrated that random errors in the dependent variable lower the correlation coefficient but do not appreciably alter the regression coefficients, while random errors present only in the independent variables and those in both dependent and independent variables, tend to decrease the magnitude of both the correlation and regression coefficients.⁶ It is obvious that in analyses of this kind any tests of significance between regression and correlation coefficients are of questionable value, since it cannot be definitely stated whether the differences observed are due to some definite assignable cause or to the inability to measure each variable accurately. This should not, however, vitiate the test of whether a correlation or regression coefficient is significant from zero, because higher values for both could reasonably be expected if it were possible to measure the variables exactly. In other words, if either coefficient is found to be significant when random errors are present, enough evidence must be accumulated within the data to indicate it over and above the influence produced by random errors.

YIELD ANALYSIS

As a preliminary step, the gross correlation coefficients for increment and reserved volume, for increment and volume of the average tree, and for reserved volume and volume of the average tree were determined and are given in table 1. The symbols used in the discussion are defined as follows:

X_1 = increment at the end of 15 years.

X_2 = reserved volume.

X_3 = volume of average tree on plot.

⁴ "Yellow pines" are ponderosa pines 200 years old and older; they have a deep-yellowish or cinnamon-brown bark. "Blackjacks" are 150 years old or less and have dark, nearly black bark.

⁵ FISHER, R. A. *STATISTICAL METHODS FOR RESEARCH WORKERS*. Ed. 3, 283 pp., illus. Edinburgh and London, 1930.

⁶ EZEKIEL, M. *METHODS OF CORRELATION ANALYSIS*. 427 pp., illus. New York, 1930. (Ch. 19.)

TABLE 1.—*Correlation coefficients for increment and reserved volume, increment and volume of average tree, and reserved volume and volume of average tree*

Variables	Gross coefficient of correlation	Gross coefficient of determination	Significance ¹
X_1 and X_2	0.7982	0.6371	Significant.
X_1 and X_3	— .3546	.1258	Do.
X_2 and X_3	— .0067	Insignificant.

¹ Determined by the aid of Fisher's table V. A., p. 176. (See footnote 5.)

The coefficients of determination, which are equal to the coefficient of correlation squared, are included because they express much more clearly the relationship between variables.

From table 1 it is evident that both reserved volume and the volume of the average tree are important factors influencing increment. Lack of intercorrelation between these factors indicates that a heavy reserved stand is not necessarily composed of large trees nor is a light reserved stand necessarily composed of small trees. As a matter of fact, the lack of significant correlation implies that the exact composition of each plot is due to chance. This lack of intercorrelation further suggested the possibility of improving the coefficient of total determination to approximately the sum of both gross determinations given in table 1, by combining the two variables X_2 and X_3 into a multiple correlation.

When this suggestion was followed out, the resulting coefficient of total determination was 0.7591 and the corresponding correlation coefficient 0.8712. Other important points in this analysis are given in table 2.

TABLE 2.—*Contribution of reserved volume and volume of average tree to volume growth*

Variables	Net regression coefficient	Standard error regression coefficient $\times 100$	Coefficient of separate determination
X_1 and X_2	0.2486 \pm 0.0162	6.5	0.6352
X_1 and X_39377 \pm .1389	14.8	.1239
Total.....			.7591

The resulting regression equation is

$$X_1 = 647.43 + 0.2486X_2 - 0.9377X_3.$$

From table 2, column 4, it can be seen that the two coefficients of separate determination are approximately equal to the corresponding gross determination coefficients in table 1. The standard errors of the two regression coefficients are included in column 2, and in column 3 they are expressed as a percentage of the corresponding regression coefficients. It is evident that reserved volume measures its influence on increment rather accurately and that the

volume of the average tree also measures its influence on increment, but to a lesser degree of reliability.

The net regression coefficient of X_1 on X_3 is negative, which means that for a given reserved volume the yield per acre tends to become

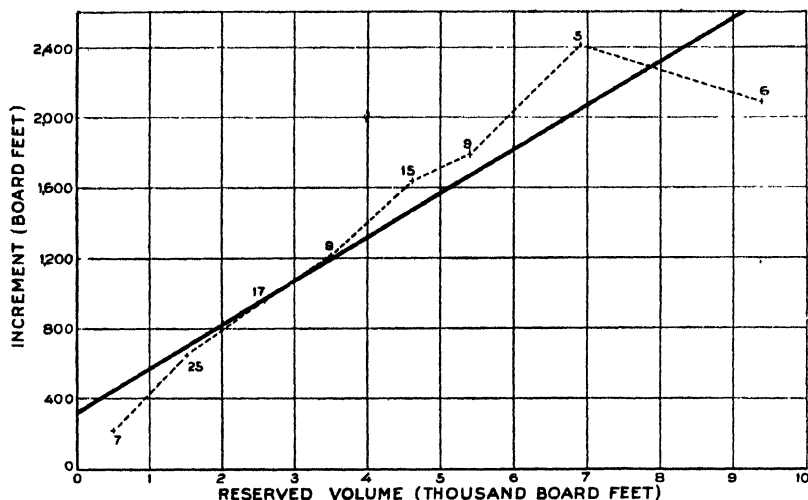


FIGURE 1.—Net regression line of increment over reserved volume with residuals.

less as the volume of the average tree increases. In other words, so far as increment is concerned it would be much more desirable to have the reserved volume divided among a relatively large number of small trees than among a few large ones.

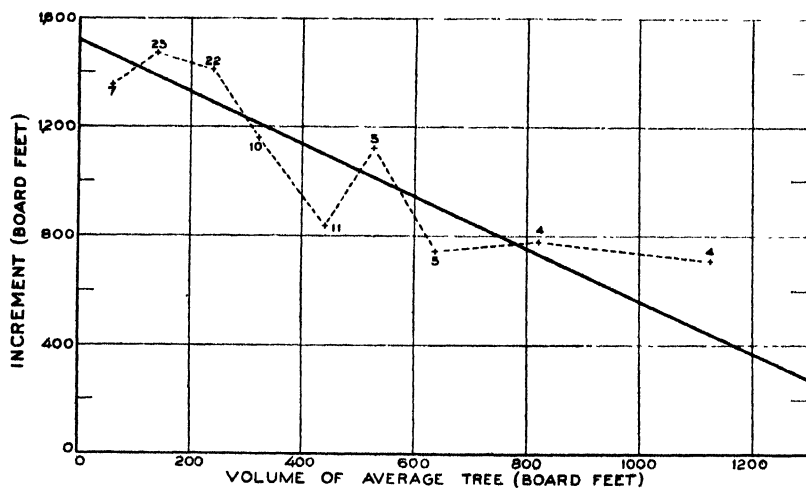


FIGURE 2.—Net regression line of increment over volume of average tree with residuals.

Up to this point the analysis has assumed that the influence of X_2 and X_3 on increment was linear. The truth of this assumption can be tested by plotting the residuals over the net regression lines

oi increment over reserved volume, and increment over the volume of the average tree. The results of these calculations are shown graphically in figures 1 and 2. There is a marked indication of

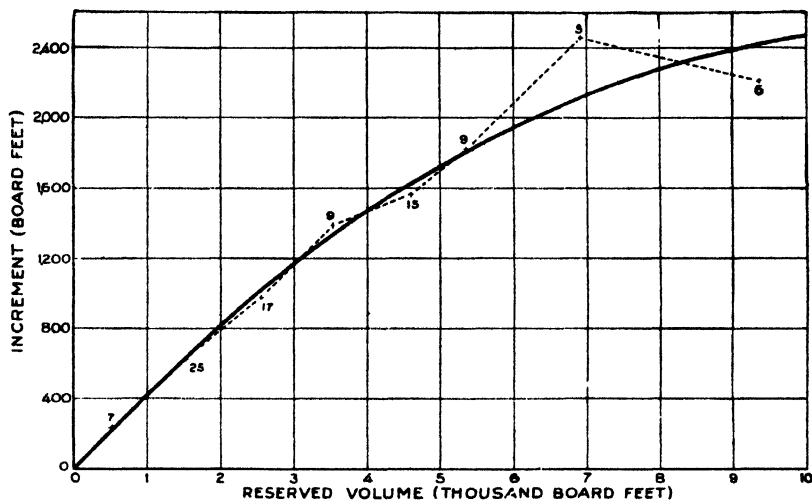


FIGURE 3.—Final net regression curve of increment over reserved volume with residuals.

curvature in figure 1 which is evident also, though it is not so pronounced, in figure 2.

By introducing curvature into the regression surface, it should be possible to further improve the coefficient of total determina-

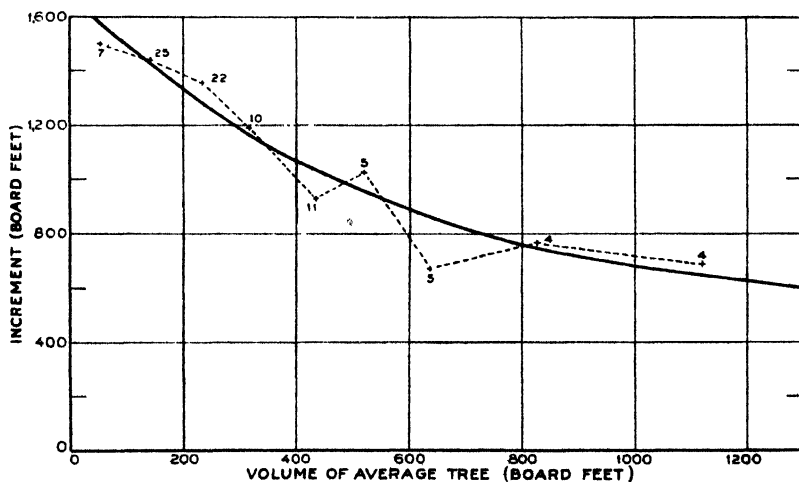


FIGURE 4.—Final net regression curve of increment over volume of average tree with residuals.

tion. This was done by the method of successive approximation.⁷ The final net regression curves are given in figures 3 and 4. They represent graphically the influence of reserved volume of increment

⁷ EZEKIEL, M. See ch. 14 of citation mentioned in footnote 6.

with the volume of the average tree held constant at its mean (351 board feet) and the influence of the volume of the average tree on increment with the reserved volume held constant at its mean (3,490 board feet). From figure 3 it is apparent that increment increases constantly with an increase in reserved volume but at a constantly decreasing rate. In figure 4 this relationship is reversed, that is, the increment becomes constantly less with an increase in the volume of the average tree, but the decrease comes at a constantly decreasing rate.

A test for joint correlation of the type where $X_1 = \theta[a + f(X_2) - f(X_3)]$ was made. The results are shown graphically in figure 5. Since

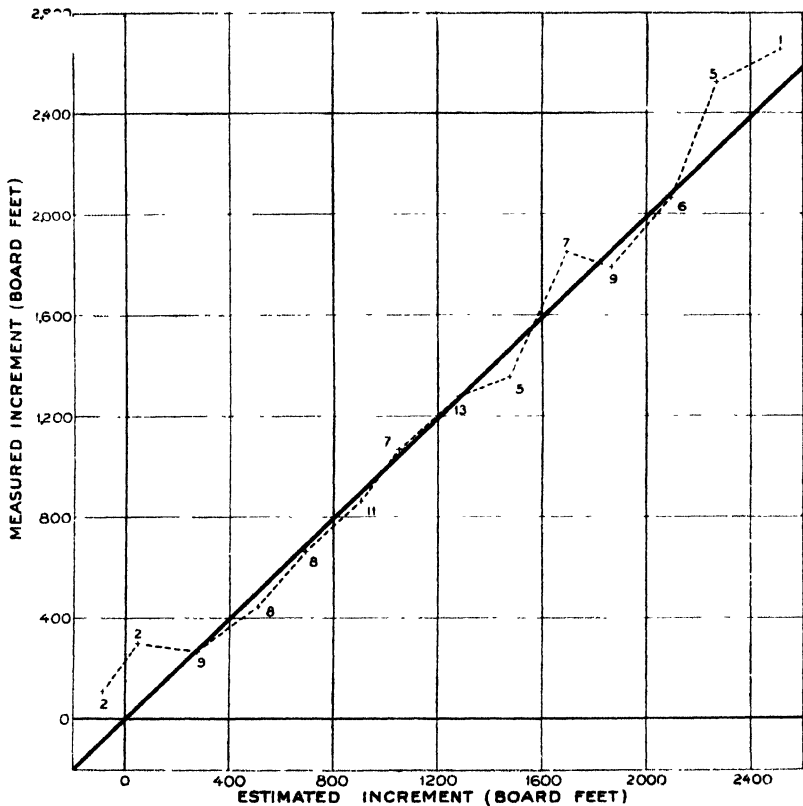


FIGURE 5.—Relation of measured to estimated increment

there was no marked indication of curvature present, it was concluded that this type of joint relationship did not exist. Further tests for joint correlation could not be made because of insufficiency of data.

Through the introduction of curvature in the regression surface it was possible to improve the correlation coefficient from 0.8712 to 0.8974 and the corresponding coefficient of determination from 0.7591 to 0.8054. From this it was concluded that approximately 80 percent of the variance of increment at the end of 15 years is associated with reserved volume and the volume of the average tree left after cutting.

MORTALITY ANALYSIS

Any determination of yield for selectively cut stands is more or less affected by loss through mortality. The amount to be deducted from gross yields due to this factor alone is subject to considerable error because of its extreme variability. Available data now indicate that if mortality continues at its present rate the annual loss from this source will be between 10 and 30 percent of the average annual growth. It has, however, been assumed that this loss will decrease with time after cutting, and table 3, which gives the mortality for each plot by 5-year periods and the mean mortality for all plots in these periods, appears to lend credence to this hypothesis.

TABLE 3.—*Loss in board feet through mortality, by 5-year periods*

Plot no 1	0-5 years	6-10 years	11-15 years	Total loss	Mean period loss
	Board feet	Board feet	Board feet	Board feet	Board feet
1.	821	0	0	821	273.67
2.	580	28	0	608	202.67
5.	0	29	0	29	9.67
7.	1,340	1,196	655	3,231	1,077.00
8.	57	50	70	177	59.00
9.	0	0	39	39	13.00
11.	0	900	0	900	300.00
12.	855	290	1,933	3,078	1,026.00
13.	0	1,180	0	1,180	393.33
15.	1,200	0	0	1,200	420.00
17.	0	1,175	0	1,175	391.67
19.	32	0	0	32	10.67
20.	29	349	31	449	149.67
23.	0	42	0	42	14.00
25.	0	31	0	31	10.33
26.	0	0	37	37	12.33
28.	632	28	0	660	220.00
29.	840	0	0	840	280.00
31.	34	902	0	936	312.00
32.	0	277	0	277	92.33
35.	0	37	0	37	12.33
37.	0	0	1,370	1,370	456.67
40.	1,396	0	0	1,396	465.00
42.	755	0	0	755	251.67
46.	778	0	0	778	259.33
47.	615	0	47	662	220.67
50.	0	42	0	42	14.00
52.	0	0	692	692	230.67
54.	0	0	645	645	215.00
55.	0	1,280	0	1,280	426.67
58.	459	0	0	459	153.00
59.	106	0	0	106	35.33
61.	603	985	0	1,588	529.33
62.	0	0	1,520	1,520	506.67
64.	0	0	25	25	8.33
67.	21	0	0	21	7.00
68.	0	0	512	512	170.67
70.	66	157	0	223	74.33
71.	0	0	88	88	29.33
74.	29	0	132	161	53.67
76.	0	194	308	499	166.33
78.	0	227	0	227	75.67
79.	58	0	0	58	19.33
82.	0	27	0	27	9.00
84.	0	0	160	160	53.33
86.	94	0	0	94	31.33
87.	0	0	52	52	17.33
89.	0	618	0	618	206.00
91.	0	449	0	449	149.67
Total.....	11,499	10,533	8,313	30,345	10,115.00
Mean.....	123.6452	113.2581	89.3871	326.29032	106.76344

1 Those of the 93 plots on which no trees died during the 15-year period are not listed

The hypothesis can be tested for the early period after cutting by the method of analysis of variance. Table 4 summarizes the analysis of the data given in table 3, the sum of squares within periods being equal to $\sum_1^{kn} (x - \bar{x}_p)^2$.

TABLE 4.—Summary of analysis of mortality data in table 3

Description	Degrees of freedom	Sum of squares	Mean square	Standard deviation	Natural log of standard deviation
Within periods.....	276	26, 214, 449. 16	94, 979. 89	308. 188	5. 7307
Between periods.....	2	57, 391. 28	28, 695. 64	169. 398	5. 1323
Total.....	278	26, 271, 840. 44	94, 503. 02	307. 413	10. 5984

¹ The value of z , determined as insignificant by the aid of Fisher's table 6. (See footnote 5.)

Where

k =number of plots used in a period

x =mortality

\bar{x}_p =mean mortality for period

n =number of periods

the sum of the squares between periods equals

$$k^2_1 S(\bar{x}_p - \bar{x})^2$$

and

\bar{x} =the general mean for all plots.

The lack of significant intraclass correlation indicates that not enough evidence has been accumulated to justify the conclusion that mortality is related to time since cutting. Although the mean mortality by periods shows a progressive decrease, the differences between means can readily be explained as being caused by errors of random sampling and not necessarily by some influence of time.

The foregoing analysis also indicates that the best estimate of the standard deviation of mortality is 307.413. With the standard deviation of mortality known, it is possible to determine the standard error of the mean from the relationship

$$s_m = \frac{s}{\sqrt{n}}$$

where

s =estimated standard deviation

s_m =estimated standard error or mean

n =number of observations.

Substituting the observed figures—

$$\frac{307.413}{\sqrt{279}} = 18.40.$$

This establishes the standard error of the mean mortality as 18.40 board feet.

The average growth for a period of 5 years is approximately 300 board feet. Should it be desired to determine the average periodic

mortality within an accuracy of ± 5 percent of the periodic growth and with odds 19 to 20 that the true mortality will be within this range, the area which must be sampled to assure this reliability can be determined from the formula—

$$n = \left(\frac{s^2}{s_m} \right)$$

where n = number of acres
 s = the best estimated standard deviation of population in question
 s_m = desired standard error.

Substituting and solving—

$$n = \left(\frac{307.413}{7.5} \right)^2 = 1,680 \text{ acres.}$$

For units smaller than 1,680 acres it is necessary to cover the area 100 percent to insure the above-stated accuracy of the mean. For larger units the percentage of area to be covered naturally becomes less.

It is sometimes possible to reduce the magnitude of the standard error of a mean by satisfactorily establishing correlation within the sample. In this case it appeared probable that mortality at the end of 15 years might conceivably be related to the amount of reserved volume and the volume of the average tree. A multiple correlation was performed, using the above-mentioned factors as independent variables, and a correlation coefficient of 0.2922 was obtained. The corresponding determination coefficient was 0.0854, which implies that only 8.5 percent of the variance of mortality is associated with the amount of reserved volume and the volume of the average tree.

The coefficient of multiple correlation in this case was so low that it appeared advisable to test it for significance. A summary of this test is given in table 5.

TABLE 5.—*Summary of test for significance of coefficient of multiple correlation between mortality at 15 years, amount of reserved volume, and volume of average tree*

Description	Degree of freedom	Sum of squares	Mean squares	One-half natural log
Regression formula.....	3	2,787,094.99	929,031.66	6.87095
Deviation from regression.....	90	29,855,077.84	331,723.09	6.35603
Value of z				10.51492

¹ Determined as significant by the same test used for z in table 4

Since the value of z was found to be significant it was concluded that reserved volume and the volume of the average tree left after cutting have some influence on mortality.

The reliability of each regression coefficient was also analyzed, and a summary of the results is given in table 6. It is evident that, while the reserved volume and the volume of the average tree are significantly related to mortality, they do not measure this relation accurately, since in both cases the standard errors are equal to approximately one-half of the corresponding regression coefficients.

In figures 6 and 7 the net regression lines of mortality over reserved volume and mortality over the volume of the average tree are plotted with the corresponding residuals. It is clear from this that an increase

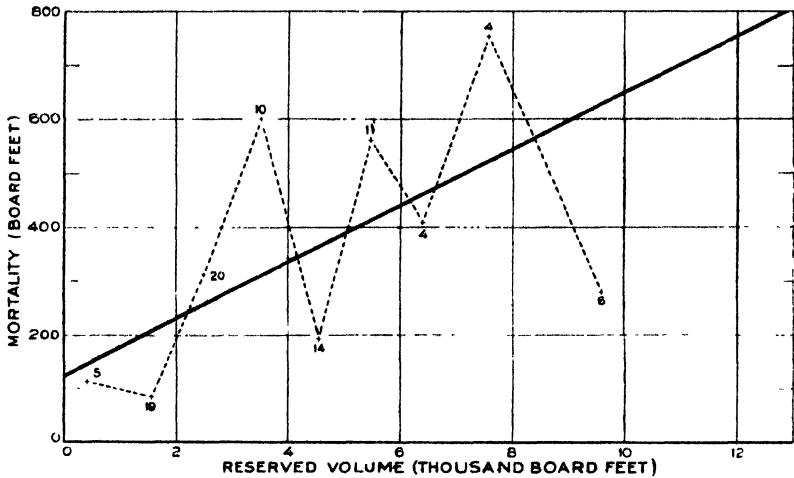


FIGURE 6.—Net regression line of mortality over reserved volume with residuals.

in either reserved volume or the size of the average tree leads to greater mortality. Casual observation of the plotted data alone indicates that this relationship is not very strong, and although a straight

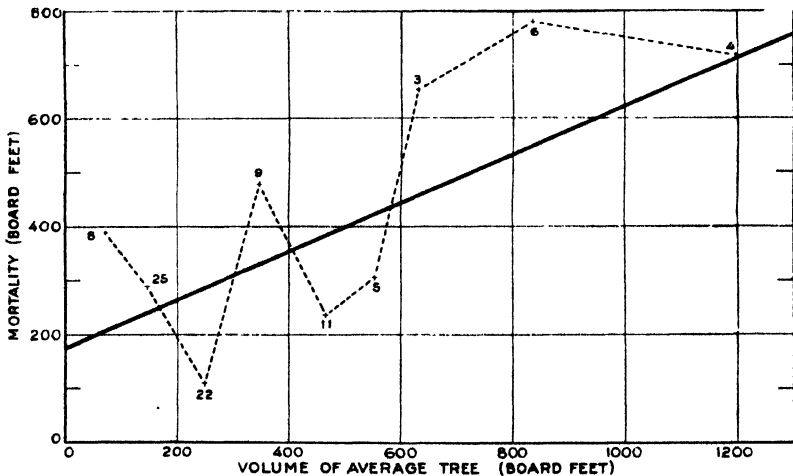


FIGURE 7.—Net regression line of mortality over volume of average tree with residuals.

line has been fitted to the data, it cannot be definitely assumed that the relationship is proportional.

TABLE 6.—*Summary of test for reliability of regression coefficients of correlation between mortality at 15 years, amount of reserved volume, and volume of average tree*

Variables	Net regression coefficients	Standard error	$\frac{\text{Regression coefficient}}{\text{Standard error}}$	$\frac{\text{Standard error}}{\text{Regression coefficient}} \times 100$
X_1 and X_2	0.0530	0.0258	2.0542	48.7
X_1 and X_34500	.2239	2.0099	49.8

SUMMARY

Analysis of factors influencing yield of timber stands indicates that the increment per acre at the end of 15 years after cutting is closely related to the volume of the reserved stand and the volume of the average tree. These two variables alone explain approximately 80 percent of the variance of increment; the remaining 20 percent is attributed to such factors as the degree of release, site differences, and differences in vigor of reserved stands.

By holding constant the influence of the size of trees left after cutting, it was found that the yield increased with an increase in reserved volume and that this increase came at a constantly decreasing rate. With the influence of reserved volume held constant, the increment decreased as the size of the average tree increased, and this decrease came at a constantly decreasing rate. This naturally follows from the fact that with a given reserved volume the amount of growing surface increases with a decrease in the average size of the trees left after cutting. Furthermore, as the size of the average tree decreases the average age also decreases, and from the nature of the curve of growth with time it follows that, in the average, more growth can be expected from a given volume of young trees than for the same volume of older trees.

The average mortality per acre for a period of 5 years was found to be 108.8 board feet with a standard error of 18.4 board feet. No significant relationship was found between mortality and years after cutting. There was, however, evidence of correlation between mortality and the two independent factors, reserved volume and volume of the average tree left after cutting, since an increase in either variable resulted in an increase in mortality. This relationship was not strong enough, however, to indicate definitely whether or not it was proportional.

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FUNGI CAUSING STAIN IN LOGS AND LUMBER IN THE SOUTHERN STATES, INCLUDING FIVE NEW SPECIES¹

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INTRODUCTION

The sapwood of many important pine and hardwood species is subject to discoloration caused by fungi. The most common stain is one of a bluish-gray hue which often occurs in spots, streaks, or patches, or which may, under suitable conditions of moisture, involve the entire sapwood area of logs and lumber. Such discolorations have been the source of serious losses to manufacturers of wood products, particularly in the Gulf States region. The present investigation was undertaken in July and August 1931 and continued through May and June 1932 to determine more definitely what fungi were chiefly responsible for such staining of wood in the southern United States. Cultural and identification studies were conducted later in the laboratory.

REVIEW OF LITERATURE

While a number of fungi have been described as causing stains in the United States, no systematic studies of their relative prevalence have been made in the field. In 1903 Von Schrenk (17)³ investigated the staining of pines in the Black Hills section and attributed the cause to a fungus which he called *Ceratostomella pilifera* (Fr.) Winter. In 1906 Hedgcock (7) published descriptions of 20 fungi which he had found capable of staining wood. In 1929 Rumbold (14) also showed, as a result of many isolations from stained wood, that there are a number of species responsible for staining. In the same year Nelson and Beal (12) gave the results of inoculation experiments in which they used three types of staining fungi which they found associated with bark beetles. The following year 2 of these 3 fungi were described by Rumbold (15), 1 never having been reported before in North America and the other being a new species. A number of the fungi mentioned by Hedgcock and Rumbold were Fungi Imperfecti, but *Ceratostomella* species have long been considered by workers in this country to be responsible for most of the staining of logs and lumber. However, Lagerberg, Lundberg, and Melin (9) state that in Sweden species of the Fungi Imperfecti were more important as stainers than were the species of *Ceratostomella*.

¹ Received for publication Dec. 26, 1934; issued July, 1935.

² The writer is indebted to Caroline T. Rumbold, of the Division of Forest Pathology, for identification of many of the fungi isolated during the first part of the investigation, and to other members of the Division staff for advice as to methods of conducting the investigations in the field. The Latin descriptions were prepared by Edith K. Cash, Division of Mycology and Disease Survey. The writer is also indebted to Nell E. Stevens, of the Division of Cereal Crops and Diseases, for identification of the species of *Diplodia*.

³ Reference is made by number (italic) to Literature Cited, p. 806.

METHODS

Freshly sawed, unstained sapwood boards were taken as they came from the sawmill and placed in newly erected stacks of lumber in the seasoning yard. Care was taken that these samples were placed where they would not dry too rapidly and thus prevent the appearance of stain. From time to time small sections were sawed from these boards and taken in paper bags to the laboratory, where an attempt was made to isolate any fungi that had grown into the wood. The transplants to agar were normally made on the same day, and almost invariably within 24 hours.

After finding that the presence of molds frequently prevented the isolation of staining fungi from lumber that had remained in the yards for 1 to 3 months or more, it was decided that, for the present study, only those stained samples which had been in the yard for 3 weeks or less would be used. The following discussion of the lumber-staining fungi and the tables giving the frequency of their occurrence are based on isolations from such freshly stained lumber samples.

At Natalbany, La., and Caryville, Fla., a chemical treatment was being used to prevent staining. There undipped boards were placed beneath the stacks of lumber to avoid direct contact with the dipped lumber. Where this was done, it was found necessary to place several test boards together, one on top of the other, in order to prevent too rapid drying.

The wood samples were from the following common tree species used for lumber in the Gulf States: Longleaf pine, (*Pinus palustris* Mill.), shortleaf pine (*P. echinata* Mill.), loblolly pine (*P. taeda* L.), red gum (*Liquidambar styraciflua* L.), yellow poplar (*Liriodendron tulipifera* L.), tupelo (*Nyssa aquatica* L.), beech (*Fagus grandifolia* Ehrh.), magnolia (*Magnolia* sp.), oak (*Quercus* sp.). At the pine mills longleaf pine was the most common species used, and at the hardwood mills red gum, oaks, and yellow poplar, probably in the order named, were the more important.

In the laboratory the wood samples from which transplants were to be made were split open and immediately placed in a culture chamber. Then small particles of wood were removed from beneath the freshly exposed surface and planted in Petri dishes containing 2-percent malt agar. These particles were removed with a "chisel forceps", which was dipped in alcohol and flamed each time after it touched the freshly exposed surface of the wood. It is possible that contamination resulted occasionally by air-carried spores, but results indicated that this was seldom the case. In making isolations from "yard-stained" lumber,⁴ which had molds fruiting on the surface, all of the outer surface was removed in a separate room, or alcohol was sometimes swabbed over the surface and flamed off. However, the results seemed about the same whether these additional precautions were taken or not.

If a sample was unstained or very slightly stained, isolations were made from near the surface, but if it was heavily stained, some were made from a greater depth and others from near the surface. In either case it was difficult to make isolation from a depth less than

⁴ By "yard-stained" is meant stain which originated after the lumber was sawed, as distinguished from log stain.

one-eighth to one-sixteenth inch without danger of obtaining surface contamination.

Most of the stained boards contained an abundant growth of perithecia of species of *Ceratostomella* on the surface. Isolations were made from these to determine whether the species fruiting on the surface were the same as those obtained from the interior of the wood. This was done by touching the droplets of ascospores, which had collected at the tips of the perithecial beaks, with a flamed platinum needle and streaking them over poured plates of malt agar. *Graphium* was frequently isolated in a similar way.

In order to determine whether the organisms causing stain in the logs were the same as those causing stain of lumber in the mill yards, the lumber was watched as it came from the sawmill and samples were taken from those already stained. Logs were also examined before they reached the sawmill and samples were taken from any that were stained.

All fungi that grew from the wood were transferred to test tubes and studied later in the laboratory. These studies were made on 2½-percent Trommer's malt extract agar and the growth rates were obtained at a room temperature of about 75° F.

OCCURRENCE OF THE FUNGI

FUNGI ISOLATED FROM PINE LUMBER

Freshly sawed pine lumber, which was placed in the mill yard to dry, showed a few small spots of stain after 3 or 4 days under humid conditions of weather. If the weather was very dry the boards remained free of stain. If the lumber was solid-piled for 2 or 3 days, either along the conveyor chain at the mill or on the runways in the yard, it became at least slightly stained in the driest weather, and if bulked for longer than 4 or 5 days the entire sapwood became badly stained.

Provided the weather was favorable for stain development, as it is much of the time in the Gulf States, the fungi were isolated from near the surface within 3 or 4 days after the lumber had been taken from the sawmill. After a period of 5 to 7 days they could usually be obtained from the center of a 1½-inch board.

The organisms responsible for the staining were found in such abundance that several species were usually present in each sample. Even when only 1 staining fungus was obtained from the interior, isolations made from the perithecia fruiting on the surface showed that 1 or 2 others were usually present. This made it difficult to associate staining with any particular organism.

The fact that several fungi were isolated more frequently than others does not necessarily prove that they are of first importance as stainers. In the first place, no pure-culture inoculations were made to determine the relative staining ability of the several species concerned. Secondly, their rate of growth in culture differs considerably, and a rapid-growing species might often appear in culture to the exclusion of the others. *Ceratostomella ips* Rumbold, for instance, grows more rapidly than *C. pilifera*, and although the two can usually be detected when growing together, it is possible that in some instances *C. pilifera* was obscured. The *Ceratostomella* hereinafter described (p. 797) as *C. multiannulata* Hedge. and Davidson, n. sp., certainly would often be suppressed by *C. ips*, *C. pilifera*, or *Diplodia natalensis* P. Evans.

The last-named species, which grows very rapidly, often suppressed all of the other staining fungi in the Petri-dish cultures.

In spite of these confusing factors, those fungi most often isolated from the interior of the stained samples seem to be responsible for most of the staining of lumber in the Southern States. This conclusion is more plausible when one considers the fact that the two fungi most frequently obtained, *Ceratostomella ips* and *C. pilifera*, are known to be severe stainers.

Table 1 includes a list of the fungi isolated from stained pine, and shows the number of times each fungus was isolated from pine lumber and logs in each locality where a study was made. In all of these localities except Caryville, Fla., sufficient samples were collected to give a good basis for determining which fungi were most frequently the cause of stain at that time. Only 6 days were spent at Caryville, which may explain in part the failure to obtain *Ceratostomella pilifera* there.

TABLE 1.—Fungi isolated from pine and hardwood lumber and logs and their relative occurrence in several localities

FUNGI FROM PINE

Fungus isolated	Number of isolations from lumber and logs in locality indicated											
	Bogalusa, La.		Urania, La., and vicinity		Laurel, Miss.		Natalbany, La.		Caryville, Fla.		Total isolations	
	Lumber	Logs	Lumber	Logs	Lumber	Logs	Lumber	Logs	Lumber	Logs	Lumber	Logs
<i>Ceratostomella ips</i>	5	13	4	2	33	6	15	1	11	6	68	28
<i>C. pilifera</i>	6	3	2	3	20	3	14	2	0	0	42	11
<i>C. multianulata</i>	0	0	0	0	5	0	0	0	0	0	5	0
<i>C. plurianulata</i>	0	0	3	1	0	2	0	0	0	0	3	3
<i>C. obscura</i>	0	1	0	0	0	0	0	0	0	1	0	2
<i>C. pini</i>	0	0	0	0	0	1	0	0	0	0	0	1
<i>C. sp.</i>	4	2	5	4	0	0	0	0	0	0	9	6
<i>Endoconidiophora moniliformis</i>	0	0	2	0	3	0	1	0	0	0	6	0
<i>Diplodia natalensis</i>	2	3	0	2	6	2	6	2	2	1	16	10
<i>Alternaria sp.</i>	0	0	3	0	10	0	0	0	0	0	13	0
<i>Helminthosporium geniculatum</i>	1	0	7	0	0	0	0	0	0	0	8	0
<i>Graphium rigidum</i>	0	1	0	0	0	0	0	1	0	1	0	3
<i>Diplodia megalospora</i>	0	1	0	0	0	0	0	0	0	0	0	1
<i>Cadophora brunneocens</i>	0	0	0	0	0	0	0	0	0	2	0	2
<i>C. repens</i>	0	0	0	0	0	0	0	0	0	1	0	1
<i>Hormonema pullulans</i>	0	0	1	0	0	0	0	0	0	0	1	0

FUNGI FROM HARDWOOD

<i>Endoconidiophora coerulescens</i>	6	8	9	8	3	5	3	8	1	1	22	30
<i>E. moniliformis</i>	0	0	12	5	2	3	3	4	4	0	21	12
<i>E. fimbriata</i>	0	0	0	0	1	0	0	0	0	0	1	0
<i>Ceratostomella plurianulata</i>	4	3	9	9	0	1	0	0	1	0	14	13
<i>C. sp.</i>	0	0	2	2	0	0	0	0	0	0	2	2
<i>Graphium rigidum</i>	4	1	9	6	0	0	0	1	0	0	13	8
<i>Diplodia natalensis</i>	6	2	2	0	1	0	0	1	0	0	9	3
<i>Leptographium microsporum</i>	2	0	0	0	0	1	0	0	0	0	2	1
<i>Hormonema pullulans</i>	0	1	1	2	0	0	0	0	0	0	1	3

It was surprising that *Ceratostomella ips* was isolated more frequently than any other species, since it had previously been considered to infect only logs infested by the *Ips* beetle. It stains the boards dark gray-black, and occasionally large short-necked perithecia

form on the stained areas. Its great abundance in lumber is probably partly due to the fact that so many logs stained by *C. ips* pass through the lumber mills. Such logs and the products of their manufacture, as lumber and sawdust, probably serve as sources of infection to the unstained lumber in the mill yard.

Ceratostomella pilifera was isolated 42 times, which indicated it to be at least second in importance. It has usually been considered the cause of most of the lumber stain, and probably it is more important than *C. ips* in some localities. The surface of wood stained by *C. pilifera* is light gray at first, due to a dense formation of white conidiophores and conidia. This light-gray surface soon disappears, leaving a dark gray-black streak of stain, which is often as dark as that caused by *C. ips*.

Diplodia natalensis was isolated 16 times from stained lumber. This species seems to be of some importance in causing stain, although the samples from which it was obtained usually contained *Ceratostomella* also. It frequently infects the boards soon after they are placed in the mill yard and grows rapidly through the wood.

The unidentified *Ceratostomella* species were probably *C. pilifera* and *C. ips*. Other species of fungi grew so rapidly in culture with them that the species could not be determined.

Endoconidiophora moniliformis (Hedgc.) n. comb., *Ceratostomella pluriannulata* Hedgc., *Helminthosporium geniculatum* Tracy and Earle, and *Alternaria* sp. are possibly of little importance in pine, since they were never definitely associated with any staining during this investigation.

The remaining species, *Ceratostomella multiannulata*, must be placed in a doubtful position as to its staining ability. In those localities where a study was made in 1932, except at Caryville, Fla., this species was found fruiting in abundance on the surface of most of the sample boards. The fact that it was isolated from the surface of 70 samples and from the interior of only 5 indicates that it seldom penetrates deeply into the wood. Its perithecia often formed and matured quickly on unstained surfaces of the boards, but wood in which it grew almost always became at least slightly stained. Whether it caused any dark staining of the wood was difficult to determine, since other species were usually present soon after its appearance. Even if it were found to be of little importance in causing stain, it must still be considered one of the common fungi inhabiting freshly sawed pine lumber in the South.

FUNGI ISOLATED FROM PINE LOGS

Some of the logs that are brought to the mills in the South already contain much stained sapwood, and although this investigation was chiefly conducted to get information on the staining of lumber, a considerable number of isolations were also made from the stained logs. It was found that *Trichoderma* sp. and other molds which were so frequently obtained from lumber stained at the mill were seldom obtained from wood that had been stained in the logs.

As is shown by table 1, *Ceratostomella ips* was found to be the species responsible for most of the log stain; it always occurred in logs that had been infested with bark beetles (*Ips* sp.). *C. pilifera* was obtained from a number of samples, and its entrance, where definitely traced, was through broken or splintered areas of the log. The two

species of *Cadophora* were obtained only at Caryville, Fla., so they are probably of little importance as stain fungi.

Since *Diplodia natalensis* is so prevalent and grows on so many substrata, it is not surprising that it was occasionally obtained from logs as well as from lumber. *Ceratostomella pini*, which is known to obtain entrance along with bark beetles, was found only once. *C. obscura*, n. sp. (described on p. 797), was isolated only twice and may not cause much staining.

NON-BLUE-STAINING FUNGI ISOLATED FROM PINE LUMBER

A number of fungi other than wood-staining species increased the difficulty of obtaining the causal discoloring organisms in culture. At Bogalusa, La., in 1931, *Trichoderma lignorum* (Tode) Harz often appeared to obscure the growth of all other fungi. It penetrated the newly sawed boards as quickly as did the staining fungi and soon invaded all of the sapwood. At Urania, La., *Trichoderma* interfered less, but other fungi such as *Helminthosporium geniculatum* and *Diplodia* hindered the growth of *Ceratostomella* species to some extent. Because of these complications, the information obtained in 1931 was considered inadequate for any definite statement as to which organisms were chiefly responsible for the staining of pine lumber.

During 1932 the work was continued at Laurel, Miss.; Natalbany, La.; Caryville, Fla.; and Bogalusa, La. At all of these places except Bogalusa, *Trichoderma* and other nonstaining fungi interfered only occasionally. However, the rapidly growing *T. lignorum* again appeared from most of the samples obtained at Bogalusa.

Other non-blue-staining fungi frequently isolated were *Pestalozzia* sp., *Fusarium* sp., various species of *Penicillium*, and occasionally species of *Aspergillus*. Several species, *Helminthosporium geniculatum*, *Alternaria* sp., and *Endoconidiophora moniliformis*, are listed as doubtful staining fungi. *H. geniculatum* was obtained only at Bogalusa and Urania, La., and was always associated with species of *Ceratostomella*.

FUNGI ISOLATED FROM SPECIES OF HARDWOOD

Table 1 shows also the fungi that were isolated from stained hardwood logs and lumber. It shows that the fungi responsible for log stain occur in about the same relative frequency in stained lumber. Here the species of Ceratostomataceae are again of much greater importance than are the Fungi Imperfecti.

Endoconidiophora coerulescens Münch is of greater relative importance as a stainer of lumber than the number of isolations would indicate. It infects the ends of the logs soon after they are cut, and continues to grow rapidly over the surface of the lumber as soon as it is stacked in the yards to season. It forms a dense black surface growth of mycelium and perithecia, and the interior of the wood is stained gray. The staining is usually more uniform than the stain in pine and not so localized in streaks or spots. *E. coerulescens* grows on most of the species of hardwoods. It was isolated from sap gum, black gum, poplar, oak, beech, and magnolia, and probably occurs on many others. The odor produced by this fungus is distinctly noticeable wherever there is abundant infection of logs or lumber.

Ceratostomella pluriannulata and *Graphium rigidum* (Pers.) Sacc. also frequently inhabit hardwood logs and lumber. They were usually

found associated with *E. coerulescens*, but grow more slowly and seem to cause much less staining than the latter. The appearance in culture of *C. pluriannulata* is very similar to *G. rigidum*, and throughout this investigation the writer was unable to identify one from the other until fruits appeared. It is not known whether they are similar in their habit of growth in the wood and in their staining qualities.

Endoconidiophora moniliformis was found very frequently on hardwoods and occasionally on pine. The fact that it was not obtained at Bogalusa is difficult to explain, for it was a very conspicuous fungus in the other localities. Factors favoring it are its very rapid growth and the fact that mature perithecia are usually formed in 2 or 3 days. It causes a dark-brown stain in fresh sapwood, but this stain seems to fade out considerably when the wood dries thoroughly. It also is frequently found growing with one or several of the species previously mentioned.

Diplodia natalensis was isolated a number of times from hardwoods, but it was usually obtained from boards that had been first stained by *Endoconidiophora coerulescens*.

The other fungi listed are probably not of very great importance. *Hormonema pullulans* (De Bary) Lagerberg and Melin was considered by Hedgcock (?) to be the cause of stain, but in the present investigation it was usually obtained from very slightly stained boards.

In isolating fungi from recently stained hardwood logs or lumber, it was found that almost no nonstaining fungi appeared to interfere with the growth of those herein listed as possible staining fungi.

SYSTEMATIC POSITION OF THE BLUE-STAINING FUNGI

CERATOSTOMACEAE

The foregoing account has been an attempt to show the relative importance of the wood-staining fungi that occur in the localities studied in the southern United States. In order that there may be little confusion concerning the identity of the species, it is essential to discuss them more fully. Complete descriptions of the well-known species are not included, but brief statements of their characteristics in culture are given.

The family Ceratostomataceae, which includes most of the important staining fungi occurring in the South, is a very unified group. The species have constant differences and may be easily identified when obtained in pure culture. In 1907 Münch (11) established the genus *Endoconidiophora*, which differs from *Ceratostomella* in that conidia are formed endogenously. This classification has not been followed by later authors. The type species, *E. coerulescens*, was the only one placed in this genus, despite the fact that *Ceratostomella fimbriata* (Ell. and Hals.) Elliott (4, 5), *C. adiposum* (Butler) Sartoris (16), and *C. paradoxa* (De Seynes) Dade (3) have endoconidia. To this *Endoconidiophora* group will be added *C. moniliformis*, which has been found by the writer to have endoconidia. These endoconidial species constitute a group of closely related fungi. This may be emphasized by the fact that *C. moniliformis* was placed by the writer in the *Endoconidiophora* group before it was examined microscopically. The production of a distinct odor seems to be a character common to all of the species, and the three species that have been isolated from wood produce two rather distinct kinds of endoconidia, the one short, thick, and barrel-shaped, and the other narrow and cylindric.

Ceratostomella ips Rumbold

Rate of growth, 24 mm in 5 days.¹

Ceratostomella ips has been adequately described by the author of the species (15). In culture it is the most rapid-growing species of

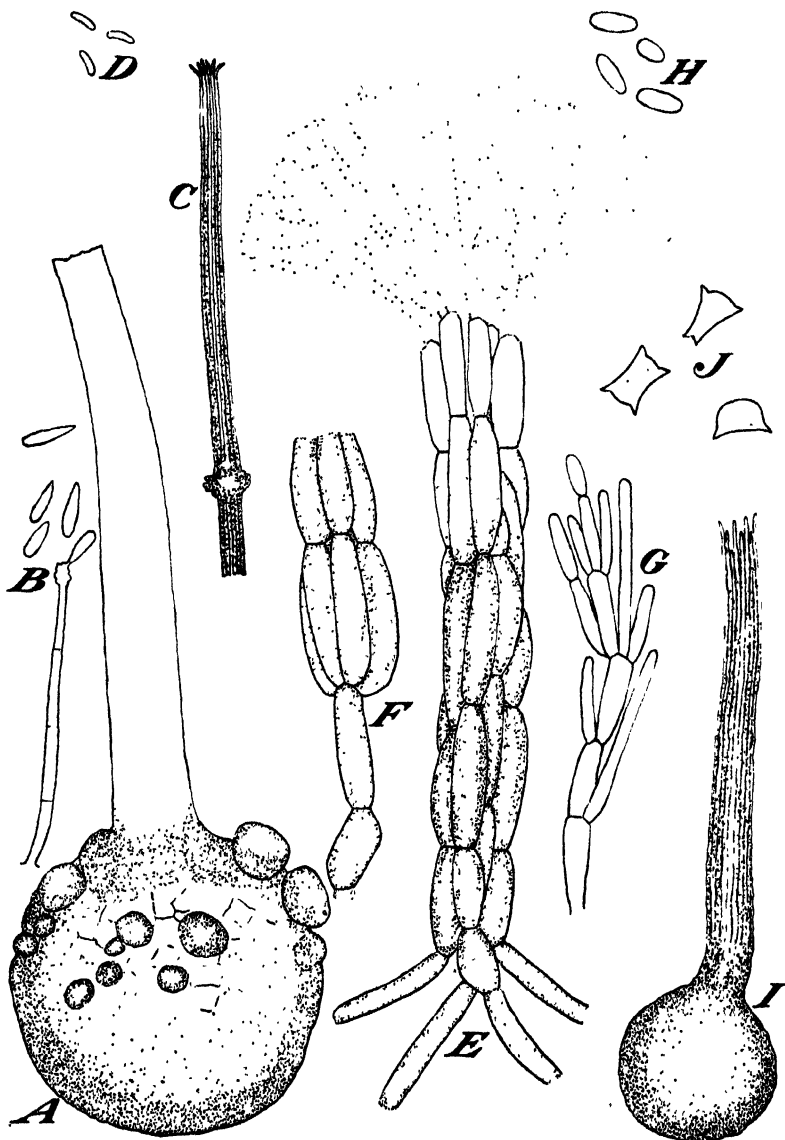


FIGURE 1.—A–D, *Ceratostomella multiannulata* Hedge and Davidson: A, Young perithecium ($\times 220$); B, conidiophore and conidia ($\times 1,000$); C, tip of beak from mature perithecium ($\times 220$); D, ascospores ($\times 1,000$). E–J, *C. obscura*: E, Small synnema ($\times 1,000$); F, base of small synnema ($\times 1,000$); G, conidiophore originating directly on mycelium ($\times 1,000$); H, conidia ($\times 1,000$); I, perithecium ($\times 220$); J, ascospores ($\times 1,000$).

¹ Growth rates, where given, indicate the radial growth on 2½-percent malt agar at a room temperature of approximately 75° F.

Ceratostomella known to infect pine lumber in the southern mill yards. This characteristic, together with its dark-brown mycelium, makes it easy to identify soon after it appears in culture. The surface of the cultures usually becomes covered by a conspicuous growth of gray-brown matted mycelium and conidiophores, but in less vigorous cultures there is sometimes no apparent aerial mycelium.

Ceratostomella pilifera (Fr.) Winter ⁶

Rate of growth, 14 mm in 5 days.

There is difference of opinion as to the validity of the name used for this fungus. Münch (11) considers it, as used by earlier mycologists, to refer to a group of fungi consisting of several distinct species. In North America, however, it has come to refer in general to the species described by Hedgcock (7) under that name.

The mycelium of *Ceratostomella pilifera* soon becomes smoke-colored and finally dark gray-black in the substratum. *C. pluriannulata* Hedgc. is the only other species studied by the writer which has mycelium of similar color and rate of growth, but it differs from *C. pilifera* in that segments or areas of the mycelium may remain entirely hyaline. The perithecia of *C. pluriannulata* also mature much earlier than those of *C. pilifera*, often forming on the hyaline mycelium as well as on the dark areas.

Ceratostomella multiannulata Hedgc. and Davidson, n. sp. (fig. 1, A-D).

Mycelio primum hyalino, brunnescente, demum obscure brunneo, supra e conidiis albo-pulverulentis; peritheciiis nigris, subdepressis, plerumque 160 μ -260 μ altis, 170 μ -275 μ latis, e cellulis obscuris globosis rugosulis; rostellis perlongis, 4-8 mm, e basi 48 μ -65 μ ad apicem 14 μ -16 μ in diam. attenuatis, 6-9 annulatis, circum ostiolum 12-16 filamentis brevibus rigidis filamentis 6 μ -12 μ longis praeditis; ascosporis hyalinis, paulo curvatis, 1.2 μ -1.4 μ \times 2.8 μ -3.8 μ , saepe conglobatis; conidiophoris hyalinis, 3 μ -5 μ latis, 70 μ -180 μ longis; conidiis 5-10 caespitosis, in apice inflato denticulate enatis, hyalinis, simplicibus, una extremitate acutis, 2 μ -3.2 μ \times 6 μ -25 μ .

Mycelium hyaline at first, becoming light brown in 4 or 5 days, and finally very dark brown, surface at first covered with a white powdery growth of conidiophores and conidia; perithecia begin to form when mycelium turns brown and mature in a day or two, are black, slightly flattened, 160 μ to 260 μ high by 170 μ to 275 μ broad, covered with dark globose cells which give a roughened appearance, necks very long, 4 to 8 mm, tapering from 48 μ to 65 μ thick at the base to 14 μ to 16 μ at the apex, multiannulate finally having 6 to 9 annulae, 12 to 16 short stiff filaments, 6 μ to 12 μ long, around ostiole; ascospores hyaline, slightly curved, 1.2 μ to 1.4 μ by 2.8 μ to 3.8 μ , often in bunches; conidiophores hyaline, 3 μ to 5 μ thick by 70 μ to 180 μ long; conidia borne at enlarged denticulate apex in groups of 5 to 10, hyaline, one-celled, pointed at one end, 2 μ to 3.2 μ by 6 μ to 25 μ .

Rate of growth, 10 mm in 5 days.

On pine lumber (nos. 59039 type, 59040, and 59041)⁷ collected at Laurel, Miss., May 1932; Natalbany, La., (no. 59042) June 1932; and Caryville, Fla., June 1932.

This species was isolated and studied by Hedgcock in 1924, but a description of it has never been published. It differs from *Ceratostomella pluriannulata* in having much longer and more pluriannulate necks on the perithecia, in having the perithecia roughened with globose cells, and in having a more distinctly brown mycelium in culture.

⁶ This fungus is possibly the same as the European species *Ceratostomella coerulea* Münch

⁷ These are numbers used by the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, and designate different strains of the fungi studied in culture. Transfers from all type cultures have been deposited in the mycological collections of the Bureau of Plant Industry.

None of the cultures obtained in 1931 was identified as this species, but samples of stained pine boards from which isolations were made in 1931 have perithecia of *C. multiannulata* on them.

Ceratostomella obscura, n. sp. (fig. 1, E-J)

Mycelio primum hyalino, ad substratum appresso, dein pallide griseo-viride, numquam obscurissimo; mycelio conidiofero fere invisibili, graphia mox formante; peritheciis tarde formatis, saepe absentibus, nigris, fere glabris, sphaericis, 90 μ –150 μ diam., rostellis 200 μ –420 μ longis, fere cylindricis, basi 30 μ –45 μ , apice 25 μ –40 μ diam., filamentis ostiolaribus setiformibus, 25 μ –35 μ longis, acutis; ascosporis membrana gelatinosa vestitis, reniformibus, 1.5 μ –2.2 μ \times 3.4 μ (membrana excludente) a hyalino pallide brunneis in massa; stipibus graphium 100 μ –700 μ altis, 15 μ –70 μ diam., saepe e hypha singula oriundis, pallide brunneis vel hyalinis; conidiis ramis penicillatis mycelii vel synnematibus distinctis enatis, hyalinis, 2 μ –3.5 μ \times 5 μ –10 μ .

Mycelium hyaline at first, appressed to substratum, becoming light gray-green in 4 to 5 days, never very dark; conidial growth at first almost invisible, graphia soon forming; perithecia slow to form, often absent, black, nearly smooth, spherical, 90 μ to 150 μ in diameter, necks 200 μ to 420 μ long, almost cylindrical 30 μ to 45 μ at base to 25 μ to 40 μ at ostiole, ostiolar filaments bristlelike, 25 μ to 35 μ long, pointed; ascospores covered with a gelatinous sheath, kidney-shaped, 1.5 μ to 2.2 μ by 3.4 μ , not including sheath, hyaline to light brown in mass; stalks of graphia 100 μ to 700 μ high by 15 μ to 70 μ thick, often originating from single hypha, light brown or hyaline; conidia borne on brushlike branches of the mycelium or on distinct synnema, hyaline, 2 μ to 3.5 μ by 5 μ to 10 μ .

Rate of growth, 17 mm in 5 days.

Isolated from pine log (59046 type) at Bogalusa, La., in June 1932, and at Caryville, Fla., (59047) June 1932.

This species was isolated only twice and probably is of little importance as a staining fungus, but it is of interest because of the *Graphium*-like imperfect stage, which is somewhat similar to that of *Ceratostomella ips*. Perithecia are seldom found. None were ever observed in single ascospore cultures, which formed graphia readily.

Ceratostomella pini Munch

Rate of growth, 25 mm in 5 days.

The writer has not made a detailed study of this species, but the culture isolated at Laurel, Miss., fits the descriptions given for it. This isolation has a very dense, conspicuous, white growth of conidiophores over the surface, in which respect it is similar to what Rumbold (15) describes as the "eastern strain" of *C. pini*.

Ceratostomella pluriannulata Hedge.

Rate of growth, 12 mm in 5 days.

This species usually occurs on hardwood logs and lumber, but was occasionally isolated from pine wood. Hedgecock (7) first described this fungus, and it was later found in Europe. The manner in which it differs from *Ceratostomella pilifera* and *C. multiannulata* is covered in the discussions of those two species.

Endoconidiophora coerulescens Munch (fig. 2, D)

Rate of growth, 33 mm in 5 days.

The microscopical and macroscopical characters of this fungus are identical with those of *Endoconidiophora coerulescens* as described by Lagerberg, Lundberg, and Melin (9). In the southern United States it was isolated only from hardwoods, while in Europe it is reported only on spruce and pine. This difference in substratum indicates that there may be a varietal difference between the European and American species. However, a fungus recently collected (in 1934) on *Pseudotsuga taxifolia* Brit. by H. E. Parks in Humboldt County, Calif., has been identified by the writer as *E. coerulescens*. This

indicates that it does occur on some coniferous hosts in North America. Another recent isolation by the writer from yellow poplar

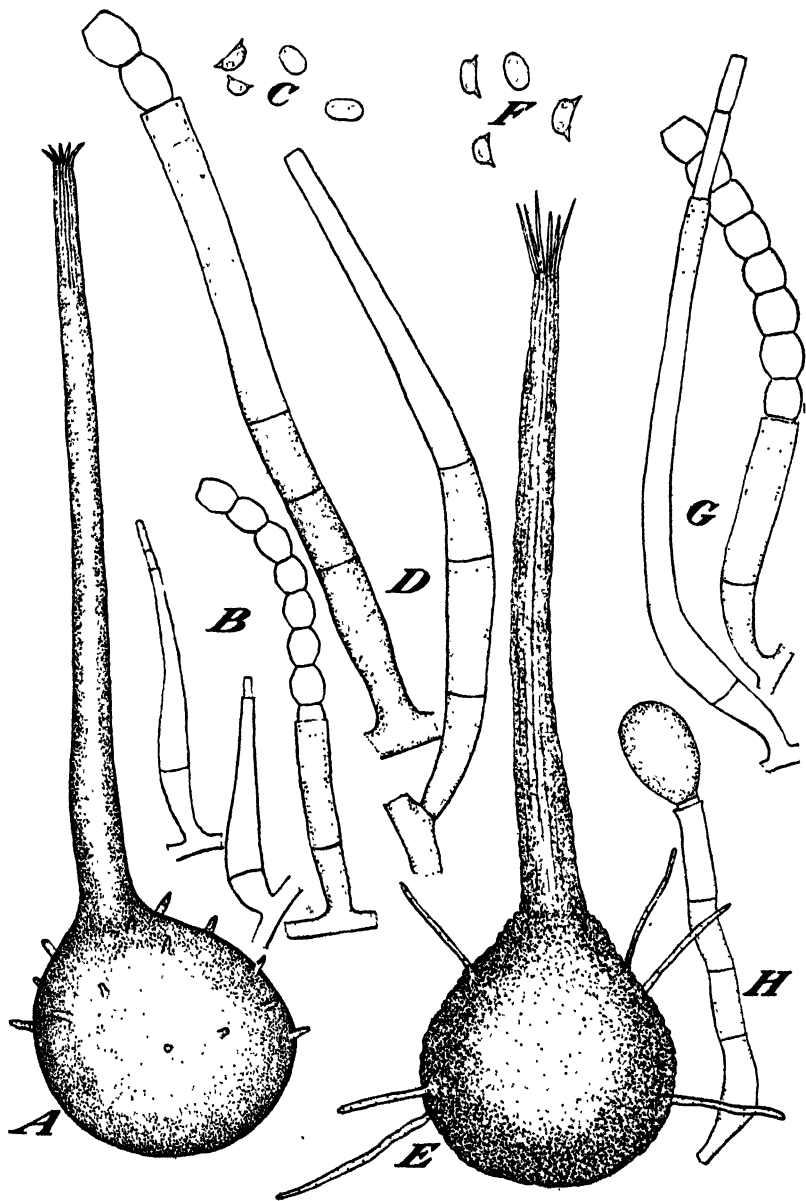


FIGURE 2.—A-C, *Endoconidiophora monilliformis* (Hedgc.): A, Perithecium ($\times 220$); B, conidiophores and endoconidia ($\times 1,000$); C, ascospores in gelatinous sheath ($\times 1,000$). D, Conidiophores of *Endoconidiophora coerulescens* Münch ($\times 1,000$). E-H, *E. ambriata* (Ell. and Hals.): E, Mature perithecium ($\times 220$); F, ascospores ($\times 1,000$); G, conidiophores and endoconidia ($\times 1,000$); H, conidiophore and brown endoconidium ($\times 1,000$).

collected in Clarendon, Va., also proves it to be more widespread in this country than the earlier study would indicate.

This species produces a very characteristic and penetrating odor both in culture and in the lumber yard. This odor differs noticeably from that produced by any of the other *Endoconidiophora* species with which the writer has worked. Lagerberg, Lundberg, and Melin (9) state that the Swedish *E. coerulescens* has an odor which is due to the production of amyl acetate.

***Endoconidiophora moniliformis* (Hedge.), n. comb. (fig. 2, A-C)**

Ceratostomella moniliformis Hedge., 1906, Mo. Bot. Gard. Ann. Rept. 17: 59-114.

Mycelium remains hyaline for 5 to 6 days, then gradually becomes light brown; odor similar to banana oil; perithecia form in 1 to 2 days and mature in 2 to 3 days, black, often elongate and lying on their sides with beaks projecting upward at an angle; 170μ to 260μ high by 150μ to 210μ broad, covered with brown bristles 3μ to 3.5μ thick by 18μ to 60μ long, necks black, 550μ to $1,000\mu$ long, 30μ to 36μ thick at the base to 14μ or 15μ at apex, ostiole surrounded by a few (8-12) hyaline filaments 15μ to 25μ long; ascospores broadly ovoid 2μ to 2.8μ by 4μ to 5μ ; endoconidiophores of two kinds, one long and attenuated to small diameter (2.5μ to 3μ) at apex and producing narrow hyaline cylindric endoconidia 2.5μ to 3μ by 6μ to 10μ , the other shorter and enlarged slightly above (4.5μ to 6μ), producing hyaline, short, thick, barrel-shaped conidia, 4.5μ to 6μ by 5μ to 7μ , in long moniliform chains.

Rate of growth, 38 mm in 5 days.

This species was the most common one found on hardwood logs and lumber, but produces a less conspicuous stain than *Endoconidiophora coerulescens* because its mycelium is hyaline or very light brown. It may be identified by the pear-shaped perithecia, which are often in a partially reclining position. The perithecia, unlike those of *E. coerulescens*, appear almost smooth under the band lens.

***Endoconidiophora fimbriata* (Ell. and Hals.), n. comb. (fig. 2, E-H)**

Ceratocystis fimbriata Ell. and Hals., 1890, N. J. Agr. Expt. Sta. Bull. 76: 14.

Sphaeronema fimbriatum (Ell. and Hals.) Sacc., 1892, Syll. 10: 215.

Ceratostomella fimbriata (Ell. and Hals.) Elliott, 1923, (Abstract) Phytopathology 13: 56.

Rate of growth, 11 mm in 5 days.

This fungus was isolated only once from a brown-stained beech board and is probably of little importance as a stainer of lumber. It is of interest, however, in that it brings to three the number of species of this genus found inhabiting hardwood lumber in the southern United States.

As in *Endoconidiophora coerulescens* (fig. 2, D) and *E. moniliformis* (fig. 2, B), this strain of *E. fimbriata* has two distinct types of endoconidia (fig. 2, G). In all three species one type is narrow and cylindrical in shape and the other barrel-shaped. Conidia of the second type have been observed, however, only in the strain of *E. fimbriata* obtained from wood.

A third type of endogenously formed spore has been commonly described for the sweetpotato strains of *Endoconidiophora fimbriata*, as illustrated by Andrus and Harter (1, figs. C and D). Only a few of these spores (fig. 2, H) were produced by the strain obtained from lumber. Unlike the first two types of conidia, this type is brown and at maturity is considerably larger in diameter than the conidiophore on which it is produced. This type of conidial formation is more nearly approached in *E. adiposa* (fig. 3, B and D) and *E. para-*

dora (fig. 3, *II*), which are also dark-spored species. This spore form has not been observed on *E. coerulescens* or *E. moniliformis*.

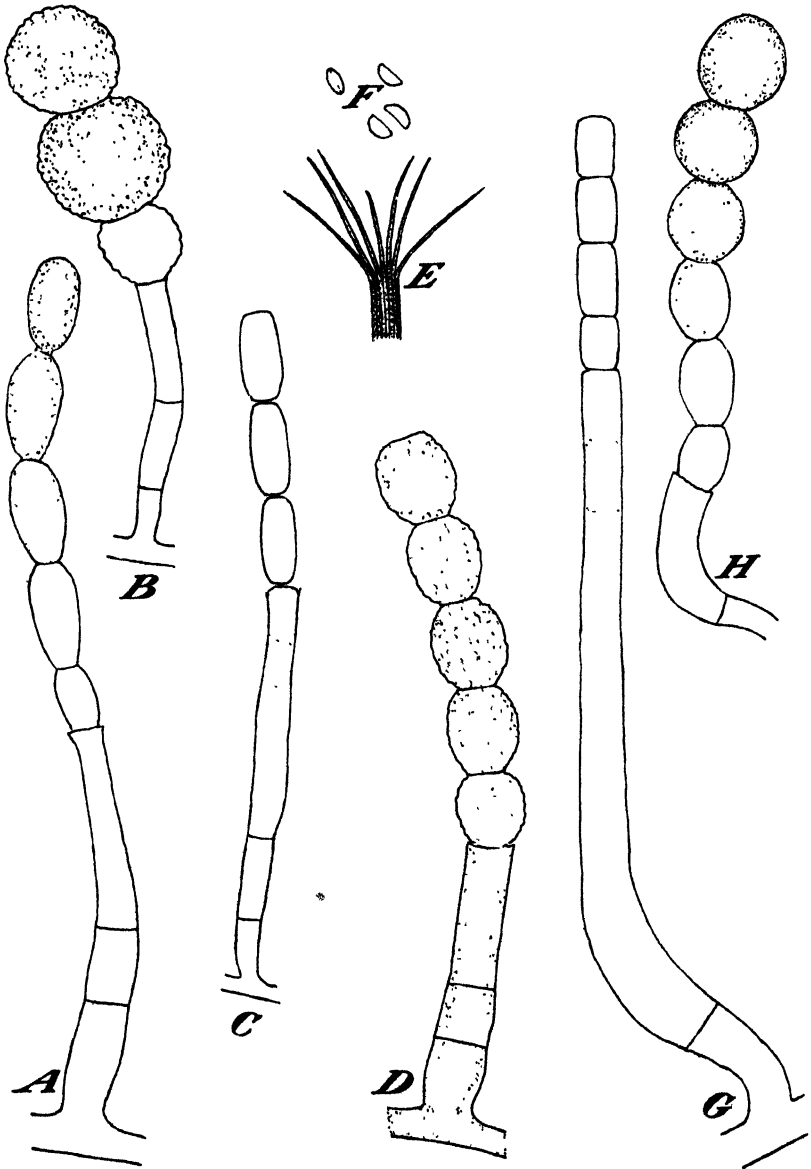


FIGURE 3.—A-F, *Endoconidiophora adiposa* (Butler): A and B, Conidiophores and endoconidia from culture isolated from *Eleocharis* ($\times 1,000$); C and D, conidiophores and endoconidia from culture isolated from basket veneer ($\times 1,000$); E, tip of perithecial beak ($\times 220$); F, ascospores ($\times 1,000$); G and H, *Endoconidiophora paradara* (de Seynes), conidiophores and endoconidia ($\times 1,000$).

The following two species were not obtained in this investigation but are included to complete the group of endoconidial species.

Endoconidiophora adiposa (Butler), n. comb. (fig. 3, A-F)*Sphaeronema adiposum* Butler, 1906, India Dept. Agr. Mem., Bot. Ser., 1, (3), 53 pp.*Ceratostomella adiposum* (Butler) Sartoris, 1927, Jour. Agr. Research 35: 577-585.

Rate of growth, 50 + mm in 5 days.

This species has been isolated from moldy basket veneer in Baltimore, Md., by Hedgecock, and from *Eleocharis tuberosa* (Roxb.) Schult., from China. The dark endoconidia are produced in great abundance. They vary in surface echinulation from the large spiny ones illustrated by Sartoris (16, fig. 1, E) to the slightly roughened ones shown herein (fig. 3, B and D). Also, great numbers of smooth spores are produced, most of which are hyaline (fig. 3, C). These conidia are not exactly comparable to the two types of hyaline conidia produced by *Endoconidiophora coerulescens* and *E. moniliformis*, although they are similar in some respects. Butler (2) has thoroughly described this considerable variation in endoconidia of *E. adiposa*. Such great variation has not been found in the endoconidia of the other species studied.

Endoconidiophora paradoxa (De Seynes), n. comb. (fig. 3, G and H)*Sporoschisma paradoxum* De Seynes, 1874, Recherches Végét. Inf. 111, p. 30.*Thielaviopsis paradoxa* (De Seynes) Höhn, 1904, Hedw., 43: 295.*Ceratostomella paradoxa* (De Seynes) Dade, 1928, Brit. Mycol. Soc. Trans. 13: 184-194.

Rate of growth, 45mm in 5 days.

The conidial form of this species was obtained by the writer along with *E. adiposa* from Chinese *Eleocharis tuberosa*. Spores of the hyaline cylindric type (fig. 3, G) were produced in great abundance, as were also the smooth, dark conidia (fig. 3, H). The latter are more nearly like those of *E. adiposa* in the way in which they are formed, but the hyaline ones are very similar to the hyaline cylindric spores produced by *E. coerulescens*, *E. moniliformis*, and *E. fimbriata*.

FUNGI IMPERFECTI

A number of additional fungi which may cause some staining of wood were occasionally isolated. They belong for the most part to unrelated groups of the Fungi Imperfecti. *Diplodia* was found to be the most common genus present in freshly stained lumber and logs. *Botryosphaeria ribis* Gross and Dug., which is very similar to *Diplodia natalensis* in culture, was obtained a number of times from somewhat weathered boards, but was never found in freshly stained lumber. *Homodendron cladosporioides* (Fres.) Sacc. was not obtained from stained wood during this investigation, although it is described by Lagerberg, Lundberg, and Melin (9) and by Hedgecock (7) as a common staining fungus.

The other imperfect forms, with the possible exception of *Graphium rigidum*, were not important as staining fungi on newly sawed lumber in the localities where this study was made. Some of them, such as *Cadophora*, may stain wood when present, but they seldom appeared in culture during the investigation. Difficulty was experienced in classifying some of these forms from descriptions. In the case of *Cadophora* it is not definitely known whether the species given herein are the same as those described by Kress et al. (8). *Cadophora brunnescens* is probably the same species as the one they illustrated

(8, pl. 20, figs. 3 and 4). Another fungus they illustrated (8, pl. 20, fig. 2) is probably another species of *Cadophora* which differs from either of the species described here.

Diplodia natalensis P. Evans

Rate of growth, 45 mm in 5 days.

Diplodia natalensis grows more rapidly in culture than any other fungus isolated from stained wood. The mycelium is white and fluffy at first, but the aerial mycelium soon becomes dark gray and the substratum black. Sclerotia containing pycnidia and spores usually form in older test-tube cultures when grown on 2½ percent malt agar, but fruits are more readily produced in flasks or test tubes of corn-meal mush.

This species is no doubt one of the important blue-staining fungi occurring in the South. It was always associated with stain in the samples of lumber from which it was isolated.

Diplodia megalospora Berk. and Curt.

Sphaeropsis ellisii Sacc., 1864, Syll. Fung. 3: 300.

Rate of growth, 45 mm in 5 days.

This species differs from *Diplodia natalensis* in having larger spores. It is a common fungus and is known to stain the stems of small infected pine trees a dark blue-gray.

Cadophora brunnescentis, n. sp. (fig. 4, E and F)

Mycelio primum hyalino, dein pallide brunnescente, demum obscure brunneo, mycelio aepophyllo brunneo, recte caespitoso, hyphis obscure brunneis, septatis, 2.5-4 μ diam., plerumque in fasciculis flagelliformibus dispositis; conidiophoris numerosissimis, uniformiter super fasciculos et hyphas singulas distributis, brevibus, 10-25 μ longis, e basi 2.5-4 μ ad apicem 1.2-2 μ diam. attenuatis, apice pallide brunneo collari munitis; conidiis endogenis, primum hyalinis et ovoideis cylindricisve, 1.2-3 μ \times 3-8 μ , dein globosis, pallide brunneis, 1.5-3 μ diam.

Mycelium, at first hyaline, becoming light brown in 3 days, then dark brown, aerial mycelium brown, erect tufted, hyphae dark brown, septate 2.5 μ to 4 μ in diameter, usually in whiplike strands; conidiophores very numerous, uniform over whiplike strands and on individual hyphae, short, 10 μ to 25 μ long by 2.5 μ to 4 μ thick at base and tapering to 1.2 μ to 2 μ above with light-brown flaring collar at apex; conidia formed endogenously, hyaline and ovoid or cylindric at first, 1.2 μ to 3 μ by 3 μ to 8 μ , later globose and light brown 1.5 μ to 3 μ in diameter.

Rate of growth, 5.5 mm in 5 days.

Isolated from pine log (59044 type) at Caryville, Fla., June 1932, and from old red gum board (59048), Urania, La., June 1931.

This species is somewhat similar to *Cadophora fastigiata* Lagerberg and Melin, but it is brown, whereas *C. fastigiata* is dark gray to black, and its conidia are more variable in size and shape than those of *C. fastigiata*.

Cadophora repens, n. sp. (fig. 4, D)

Mycelio in agar (malt) primum hyalino, dein pallide brunnescente, demum obscure brunneo, ad substratum appresso, raris cirrhis aepophyllis exceptis, hyphis brunneis, 2-4.5 μ diam., saepe in fasciculis dispositis; conidiophoris plerumque in ramis brevibus caespitulosis, parvis, 10-15 μ longis, e basi 2-3 μ ad apicem 1-1.5 μ attenuatis, hyalinis vel pallide brunneis; conidiis endogenis, hyalinis, cylindricis, interdum curvatis, 1-1.5 μ \times 2.5-8 μ .

Mycelium, on malt agar, hyaline at first, becoming light brown in 5 days and finally dark brown, appressed to substratum except for a few aerial tufts of hyphae; hyphae brown 2 μ to 4.5 μ in diameter, often in strands; conidiophores usually grouped on short branches, small, 10 μ to 15 μ long, tapering from 2 μ to 3 μ at base to 1 μ to 1.5 μ at apex, hyaline or light brown; conidia endogenously formed, hyaline, cylindric, sometimes curved, 1 μ to 1.5 μ by 2.5 μ to 8 μ .

Rate of growth, 22 mm in 5 days.

Isolated from pine log (59045 type), Caryville, Fla., June 1932.

This species is distinct because of its very rapid growth in culture and the grouped conidiophores. The conidiophores are usually

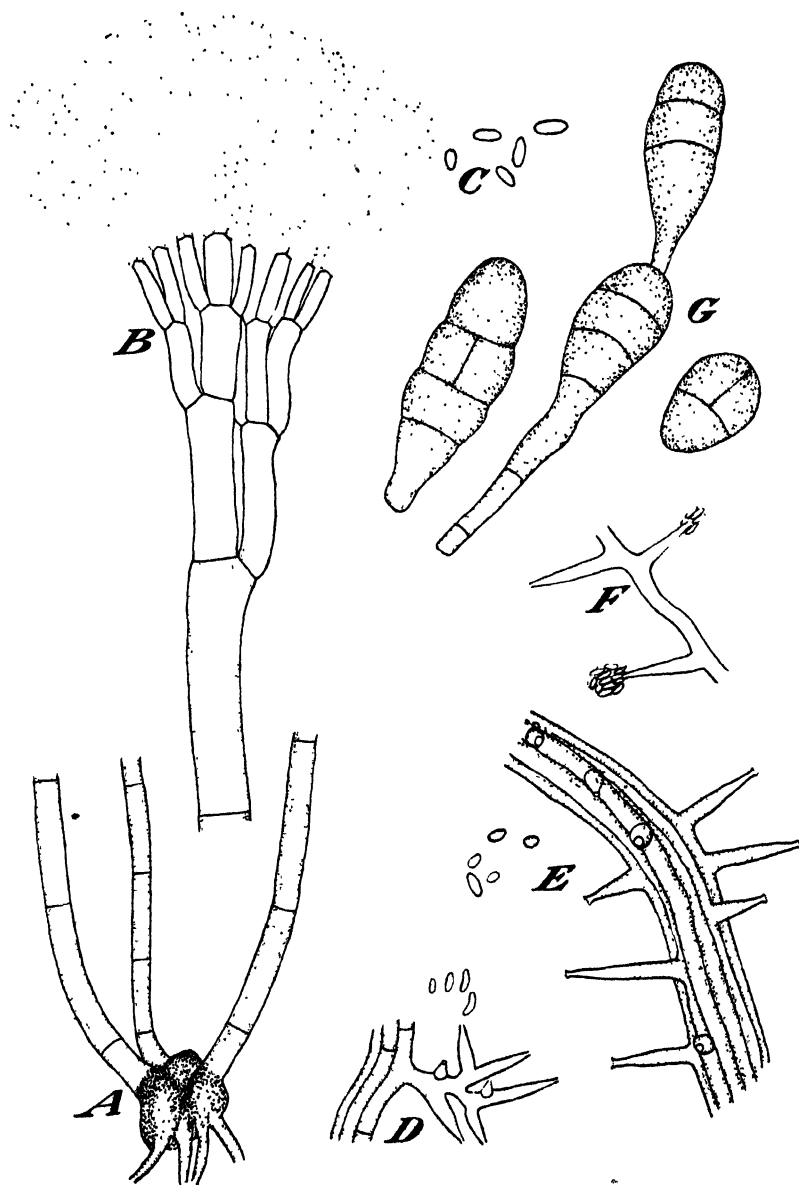


FIGURE 4.—A-C, *Leptographium microsporum*: A, Basal portion of group of conidiophores ($\times 220$). B, apex of sporulation conidiophore ($\times 1,000$). C, conidia ($\times 1,000$). D, Conidiophores and conidia of *Cadophora repens* ($\times 1,000$). E and F, *Cadophora brunnescens*: E, Ropelike strand bearing conidiophores ($\times 1,000$); F, single hyaline hyphae bearing conidiophores ($\times 1,000$). G, Conidia of *Allernaria* sp. ($\times 1,000$).

hyaline or very light brown at the base and have no distinct flaring collar at the apex. The spores are always hyaline.

Leptographium microsporum, n. sp. (fig. 4, A-C)

Mycelio primum hyalino, byssoideo, demum obscure griseo et densissime intertexto, conidiophoris tenuibus dense tectis; conidiophoris longe pedicellatis, 5μ - 10μ diam., 125μ - 500μ longis, apice e capitulo penicillato praeditis, ramis terminalibus hyalinis, apice conidia muco-conglutinata gerentibus; conidiis parvis, 0.9μ - $1.5\mu \times 2.5\mu$ - 3.5μ , hyalinis, interdum leniter curvatis.

Mycelium hyaline, cottony at first, finally dark gray and densely matted, covered with dense formation of fine conidiophores; conidiophores long-stalked, 5μ to 10μ in diameter by 125μ to 500μ long, with a brushlike head at apex, terminal branches hyaline and bearing conidia at their apex which cling together in a mucous mass; conidia small, 0.9μ to 1.5μ by 2.5μ to 3.5μ , hyaline, sometimes slightly curved.

Rate of growth, 7.5 mm in 5 days.

Isolated from beech board (59043 type) at Laurel, Miss., May 1932, and from red gum board (59050) at Bogalusa, La., June 1931.

The conidiophores of this fungus are similar to those of *Leptographium penicillatum* Grosmann (6) in manner of branching, but there are usually 2 to 5 formed together at rhizomelike nodes as in *Scopularia venusta* Preuss (10, p. 745).

The boards from which this fungus was obtained had previously been stained by *Endoconidiophora coerulescens* and also contained *Graphium rigidum*.

Hormonema pullulans (De Bary) Lagerberg and Melin

Hormiscium gelatinosum Hedgc., 1906, Mo. Bot. Gard. Ann. Rept. 17: 59.

Rate of growth, 11 mm in 5 days.

This fungus more nearly fits the description given by Robak (13) for *Hormonema pullulans*. Cultures isolated by the writer formed no aerial mycelium but remained entirely smooth on the surface of the substratum.

This species seemed to cause little stain in the fresh lumber from which it was isolated. It was found in slightly stained old boards and may be the cause of considerable staining in such material. It was, of course, difficult to determine definitely just which or how many fungi were responsible for this latter stain.

Helminthosporium geniculatum Tracy and Earle

Rate of growth, 9 mm in 5 days.

This is a slow-growing fungus which was obtained from pine boards placed in the yard for only a few days. It is possible that infection occurred when sample boards were dropped on the grass that grew at the sides of the lumber piles. Species of *Ceratostomella* were always present in the boards from which it was obtained.

This fungus is characterized by its curved brown spores and cylindric sclerotia, both of which are produced in culture.

Graphium rigidum (Pers.) Sacc.

Rate of growth, 12 mm in 5 days.

This species of *Graphium* is very common on hardwood logs and lumber, where it is usually associated with *Ceratostomella pluriannulata* and *Endoconidiophora coerulescens*. In culture the mycelium is very similar in appearance to *C. pluriannulata*, but it fruits readily, so was not confused with that species.

Alternaria sp. (fig. 4, G)

Rate of growth, 14 mm in 5 days.

White fluffy mycelium at first, becoming mouse-gray and the substratum dark gray-black; conidiophores form on the older mycelium, usually on or near the substratum, dark brown, 3μ to 4.5μ thick, long or short, septate; spores varying

greatly in shape and size, 8μ to 15μ by 16μ to 35μ , dark brown muriform. Usually 3 or 4 cross walls with one longitudinal septum through the larger part of the spore.

At Laurel, Miss., this fungus was frequently one of the first to be cultured from the boards, but it could never be definitely associated with stain.

SUMMARY

A study made in several localities of the southern United States showed that species of *Ceratostomataceae* were responsible for most of the initial blue stain in lumber and logs. *Ceratostomella ips* and *C. pilifera* were most frequently isolated from recently stained pine logs and lumber, and *Endoconidiophora coerulescens*, *E. moniliformis*, and *Ceratostomella pluriannulata* from stained hardwood logs and lumber.

A number of Fungi Imperfecti were also obtained from stained lumber, but *Diplodia natalensis* and *Graphium rigidum* were the only ones of this group which seemed to be of considerable importance.

Two new species of *Ceratostomella* were obtained from pine. One of these, *C. multiannulata*, was the most common species found fruiting on lumber but seemed to cause little discoloration in the interior of the wood. *C. obscura*, the other new species, was obtained only twice from stained pine logs.

The genus *Endoconidiophora* has been retained for those species of *Ceratostomella* having endoconidia. Three species were isolated from lumber and two others were studied and placed in this genus.

Three Fungi Imperfecti, *Cadophora brunnescens*, *C. repens*, and *Leptographium microsporum*, obtained from stained wood, are described as new species. They do not seem to be of much importance as wood-staining species.

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EFFECT OF ANNUAL GRASS FIRES ON ORGANIC MATTER AND OTHER CONSTITUENTS OF VIRGIN LONGLEAF PINE SOILS¹

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INTRODUCTION

The winter burning of dead grass left unconsumed by grazing animals from the growth of the previous season is a world-wide practice of very ancient origin, particularly in humid regions where uncut grass does not cure into palatable winter forage. By the use of grass fires the Indians maintained open grazing lands for the bison and pronghorn antelope, the largest herds of grazing animals that the world has known. This practice of the Indians was considered beneficial to the land and was continued by white men, particularly in the humid longleaf pine region of the South, as a means of keeping down the underbrush and improving the pasturage for cattle. In the longleaf pine region a large part of the virgin soil is burned over each year. Hilgard (10, p. 495),³ writing of the longleaf area of southern Mississippi, stated:

"The land * * * affords but indifferent pasturage, except the first season after burning-over; probably because of the effect of the minute amount of ashes so added."

Referring to the effect of grass fires on forest growth, Harper (8, p. 668) in 1913 stated:

* * * they return immediately to the soil the mineral plant food stored up in the leaves. The amount of the available plant food in the soil of the pine forests is usually rather limited, and these frequent fires thus enable the pine to do business on a small amount of capital, as it were.

The literature does not seem to record any conflict with the empirical observations of the users of grass fires until recent years, when objections to the use of fires were made on the theoretical basis that the fires destroy organic matter and nitrogen to the detriment of soil fertility. The general unsupported argument against the use of fire was stated by Van Hise (24, p. 238) in 1910, as follows:

The fires do not simply confine themselves to the timber, but they burn the humus in the soil itself. Frequently, after a great forest fire, and especially if the fires run over the same area two or three times, there is left of the soil, sand, and other minerals, but little of the original organic material.

Mattoon (16, p. 48) in 1931, states:

The leaves, or "straw", from pines contain considerable nitrogen and small amounts of phosphoric acid and potash. A ton might contain these essential fertilizing elements to the value of \$2 to \$4. An unburned pine woods may have as much as 10 to 15 tons [per acre] of leaves and other organic matter.

¹ Received for publication Feb. 18, 1935; issued July, 1935. The data reported were obtained in a cooperative grazing and reforestation experiment conducted at the McNeill Experiment Station, McNeill, Miss., by the Bureau of Animal Industry and Plant Industry and the Forest Service of the U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station.

² The writer is indebted to H. R. Reed, formerly of the Bureau of Plant Industry, who spent considerable time in making the final legume counts. Acknowledgment is made also to W. F. Hand, State chemist of Mississippi, for analyses of soils and for determinations of the protein and ash content of grasses.

³ Reference is made by number (italic) to Literature Cited, p. 821.

However, Mattoon gives no basis for his statements and does not indicate any way in which organic matter and nitrogen, on top of the virgin soil, might be recovered in the soil for the use of growing plants.

RELATED INVESTIGATIONS

Alway and Rost (2), from experimental work in 1918-19, found that burning did not influence the immediate fertility of the mineral soil and concluded that any loss in productivity would depend on the loss of nitrogen contained in the forest litter, since the mineral elements are returned immediately to the soil by fire. In later experiments Alway found that burning the forest floor as compared with plowing under the natural litter had no significant effect on crop production either immediately following (1) or over a series of years.⁴

Greene (7), in 1929, showed that cattle made 44.4 percent greater gains on burned native grass pastures than on similar unburned pastures over a period of 4 years.

The literature in regard to the accumulation of humus and nitrogen in cultivated soils and their effect on crop production is voluminous. Such literature, however, is concerned exclusively with the problem of green manuring, or the turning under of plant residues, and does not consider the problem of humus and nitrogen or their accumulation in soils that have not been plowed and are to be handled as virgin soils in forests or pastures where vegetation is either burned or left to decay where it accumulates on top of the soil.

The emphasis placed by popular agricultural literature on the value of organic matter plowed under, without regard to the quality or quantity of the material to be turned under, has, no doubt, created a tendency to overestimate the fertilizing value of vegetative growth on virgin soils. Moreover, the theoretical grounds on which deductions may be based are not always realized in practice. Such a concept for virgin soils leaves out of consideration the methods by which nature incorporates plant residues with the soil.

The importance of organic matter in the soil has always been recognized, but a sharp distinction must be drawn between organic matter in the soil and on top of the soil. According to Pieters (19), organic matter on top of virgin soils may be incorporated with the soil by the action of water or glaciers, by rodents, insects, earthworms, and micro-organisms, and by the sharp hoofs of grazing animals, but chiefly by the decay of plant roots.

Soil nitrogen in any form is derived originally from the gaseous nitrogen in the air and is a rather unstable and transient material, the gains and losses of which are perhaps not yet fully explained. Small quantities of gaseous nitrogen, in forms available to plants, are added to the soil by rainfall and by free-living micro-organisms, but the chief source of increase is through the action of the micro-organisms associated with legumes. Finnell and Houghton (6) found that 15.45 inches of rainfall in 1930 added 1.42 pounds of nitrogen per acre. No measure of the quantity of nitrogen per acre fixed by free-living organisms seems to have been obtained, and the effect of this class of organisms appears to be almost purely speculative.

⁴ Unpublished information.

Nitrogen is an essential element of plant growth, and the nitrogen content of the soil is closely associated with the organic-matter content. Plants other than legumes, grown and left in place, do not add nitrogen to the soil but transform soil nitrogen into organic nitrogen with a loss to the soil in the process. Because of the known ability of legumes to store a considerable quantity of nitrogen extracted from the air, in both the root and aboveground portions of the plant, the maintenance or increase of nitrogen in virgin soils may depend largely on the character of the plant population.

In annual legumes one-fourth to one-third of the total nitrogen has been found by the Mississippi Agricultural Experiment Station to be in the portion of the plants below ground,⁵ and in some perennial legumes nearly half of the nitrogen is in the underground portions (19, p. 74).

Lyon and Bizzell (15), by lysimeter experiments, for a period of 15 years, in which the nitrogen added by rainfall and manure and removed by the crops and in the drainage water was carefully measured, found that in tanks containing growing legumes there was an increase of nitrogen in the soil equivalent to about 60 pounds per acre per year. When the tanks did not produce crops for 10 years and then grew nonlegumes for 5 years, the loss of nitrogen from the soil was at the rate of about 25 pounds per acre per year.

Sievers and Holtz (21) have shown that both the organic-matter and nitrogen increase in soils is influenced by the nitrogen-carbon ratio of the plants grown on the soil or turned under.

In the mature stages of plants it has been shown by LeClerc and Breazeale (12) and others that leaching of the mineral elements from plants in place occurs in considerable quantities, but that leaching of nitrogen is relatively unimportant.

Soil nitrogen is decreased by the removal of crops which contain nitrogen, by conversion into ammonia gas and gaseous nitrogen through the action of micro-organisms, both bacteria and fungi, and by leaching.

Heck, Musbach, and Whitson, as reported by Clark (4), found that the loss of organic nitrogen from manures on the ground in free circulation of air is much more rapid than below ground. The same relation between nitrogen in the aboveground and below-ground portions of mature plants would be indicated.

Nitrate nitrogen, the form most available as a plant nutrient, is readily soluble and is quickly leached away if not taken up by growing plants. Lyon and Bizzell (14) have shown that the loss of nitrogen by leaching was 17 times as much on uncropped land as on cropped land. Because of the loss of nitrogen both as ammonia and as nitrate on uncropped land, the use of winter cover crops as well as summer growing crops is generally advocated to conserve nitrogen on tilled soils in humid areas with mild winters. On virgin soils a constant maximum growth of herbage is indicated to conserve soil nitrogen.

The foregoing references underlie the concept derived from the experimental data to be presented. Although fire has, no doubt, been the most violent if not the most active chemical reaction present on the soil of the virgin longleaf pine region and is known to have a very active influence on the plant population of virgin soils, no data other

⁵ Unpublished information.

than the limited work cited have been found showing the influence of frequent or annual burning on the fertility of the soil, as compared with protection from fire.

EXPERIMENTAL PROCEDURE

A tract of 320 acres of virgin land near McNeill, Miss., reproducing to longleaf pine about 20 years after the virgin timber had been removed, was fenced and divided into four experimental areas in 1923. Previously the land had been unfenced open range, subject to annual or periodic burning and lightly grazed by cattle. Since the fenced area was a part of a large area of open land with no natural barriers to fire or grazing, it is believed that the areas as divided had received uniform treatment before being fenced. This area is typical of the rolling longleaf pine hills of southern Mississippi. It has an elevation of 230 feet, and is well drained, as are the soils of the heavier type in longleaf pine areas.

Two areas of 150 acres each were grazed, one of which was burned annually during winter or early spring, beginning in 1923. Two 10-acre areas were not grazed, and one of these was burned annually. A detailed soil map of the area, made by the Bureau of Chemistry and Soils of the United States Department of Agriculture, was used as an aid in establishing the experimental areas and plots.

No soil analyses were made at the beginning of the experiment, since the studies undertaken concerned mostly problems of forage production. Soil analyses were made later in seeking an explanation of differences in forage growth under different treatments. Detailed studies of the variations in the plant population were made annually.

To determine the effect, on the soil, of burning the grass annually over a considerable period of time, analyses were made of soil samples collected on April 23, 1929, about 3 months after growth had begun in the spring and during the season of flush growth, and on January 10, 1930, during the dormant period. As it was recognized that individual samples might vary in chemical composition within a radius of a few feet, five random samples were taken to a depth of 6 inches for each soil type, and these five were mixed thoroughly to form a composite sample.⁶ In addition to the principal studies relating to organic matter and nitrogen content of the soil, other tests dealt with density of plant growth, moisture content, and micro-organisms in soil from burned and unburned areas.

EXPERIMENTAL RESULTS

ORGANIC MATTER AND NITROGEN

Table 1 shows the content of organic matter and nitrogen from moisture-free samples of soil on the dates mentioned.

In samples taken in April the average organic-matter content of soil from the burned areas was 3.17 percent as compared with 2.59 percent for the unburned areas, or a ratio of about 1.2:1. The corresponding figures for nitrogen content were 0.047 and 0.055, or a ratio of about 0.9:1. Although the samples collected in April showed a variation in organic matter in favor of the burned areas in each instance, the proportion of nitrogen to organic matter was sometimes

⁶ Soil samples were collected by the Southern Forest Experiment Station of the U. S. Forest Service. Soil analyses were made by W. F. Hand, State chemist of Mississippi.

TABLE 1.—Organic matter¹ and nitrogen in composite samples of moisture-free soils taken Apr. 23, 1929, and Jan. 10, 1930, from soils subjected for 7 and 8 years, respectively, to the treatment indicated

APRIL 23, 1929, SAMPLES

Soil type ² (fine sandy loam)	Grazed area				Ungrazed area			
	Burned		Unburned		Burned		Unburned	
	Organic matter	Nitrogen	Organic matter	Nitrogen	Organic matter	Nitrogen	Organic matter	Nitrogen
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Orangeburg	2.41	0.033	2.30	0.050	2.47	0.045	2.33	0.040
Norfolk	3.36	.046	2.62	.050	2.67	.050	(3)	(3)
Ruston	4.81	.053	2.40	.050	(3)	(3)	2.98	.050
Kalma	3.29	.053	2.89	.090	(3)	(3)	(3)	(3)
Average	3.47	.046	2.55	.090	2.57	.047	2.65	.045

JANUARY 10, 1930, SAMPLES

Orangeburg	3.62	0.06	2.26	0.04	4.21	0.07	2.85	0.05
Norfolk	3.87	.06	2.87	.05	3.88	.07	(3)	(3)
Ruston	3.47	.07	2.41	.04	(3)	(3)	2.24	.04
Kalma	6.90	.10	3.15	.07	(3)	(3)	(3)	(3)
Average	4.46	.07	2.67	.05	4.05	.07	2.54	.04

¹ Analysis made by ignition method.

² For description see soil survey by Smith and Carter (22).

³ No sample taken.

in favor of the burned areas and sometimes in favor of the unburned areas. It is known that the available nitrate nitrogen is taken up rapidly in the early flush of forage growth. A simple calculation from the known yields and analyses of the forage shows that the difference in nitrogen content for different areas, after the growing season had advanced approximately 90 days, may have been sufficient to be reflected in the analyses of the soil.

The soil samples taken January 10, 1930, during the dormant period of plant growth for the region, were repetitions of samples taken April 23, 1929, during the season of flush growth of the native vegetation of the region and after about 3 months of the growing season.

In the soil samples taken in January, the variation in organic matter and nitrogen from burned and unburned areas during the dormant period for plant growth, as shown in table 1, was reasonably constant. The average content of organic matter in all samples from burned areas was 4.32 percent as compared with 2.63 percent for the unburned areas, or a ratio of about 1.6:1. The corresponding average differences in nitrogen content were 0.072 percent and 0.048 percent, or a ratio of 1.5:1. The percentages of organic matter and nitrogen were in all cases higher on the burned areas. The highest percentage of organic matter from any unburned sample did not equal the lowest from any burned sample.

The differences in January between the burned and unburned areas in both organic matter and nitrogen were of such magnitude as to be significant. A difference in weight of 0.01 percent in a moisture-free sample of soil to a depth of 6 inches would amount to approximately 155 pounds per acre, according to the weights of Mississippi soils as

given by Logan (13). In terms of nitrogen for soils that are deficient in this element, a difference of 0.001 percent, when analysis is made to the third decimal point, is significant. The average difference in percentage of nitrogen was 0.024 percent (0.072–0.048), which represents approximately 400 pounds of nitrogen per acre, in favor of burning. This difference is equal in amount to the nitrogen in an application of approximately 2,400 pounds of nitrate of soda, the common nitrogenous fertilizer of the region.

The conclusion seems warranted that sampling soils for nitrogen during the growing season would give unreliable comparisons for soils that are producing crops varying widely in quantity and quality, especially for soils deficient or low in nitrogen, where the current supply of available nitrogen is a limiting factor for plant growth.

With respect to the soil samples taken at a time of year when plant growth is practically dormant, and over a large area which previously had been subject to uniform treatment, it is reasonable to conclude that the differences in soil analyses were due directly or indirectly to differences in the treatment while under control.

GROWTH OF FORAGE

Throughout the course of the experiment, the growth of forage in the different areas was observed annually on a series of more than 50 rectangular plots of 0.01 acre each.

In 1930, after 8 years of annual burning as compared with complete fire protection over the same period, weights were taken of forage on burned and unburned areas where neither had been grazed (table 2). Two of the 0.01-acre plots were used for each of the two predominating grasses, *Andropogon scoparius* (little bluestem) and *A. tener*, where they were growing in practically pure stands, and two plots for mixed stands. These were selected in the open, away from shade and only a few yards apart on each side of the fire line so that all conditions would be the same except the factor of fire.

TABLE 2.—Yield per acre¹ of the predominating grasses on ungrazed areas, burned and unburned, for 8 years

Kind of grass	Green plant per acre on—	
	Burned area	Unburned area
	Pounds	Pounds
<i>Andropogon scoparius</i>	5, 121	3, 623
<i>A. tener</i>	6, 957	1, 206
Mixed stand.....	5, 749	2, 415
Average.....	5, 942	2, 415

¹ The grasses were clipped about one-half inch above the ground.

The differences in weight on the burned and unburned areas were due chiefly to the reduction of stands (smaller number of plants per acre) on the unburned plot, although there was a reduction in the vigor of growth on this plot as shown by the characteristic spindly growth of plants that are required to grow through a mulch that reduces light in the early stages of growth. The average of the maximum heights

of the plants on the plots studied in the burned area was 20 inches. The corresponding figure for the unburned area was 30 inches. From these studies it appeared that the density of the grass growth on areas that were not burned was reduced from year to year by the smothering or mulching effect of the dead debris of the grasses themselves. On unburned areas where the grasses were further blanketed by the "strawfall" from pine saplings, forage growth was almost completely eliminated in the course of a few years. This reduction in forage growth through the action of dead debris on unburned land is cumulative from year to year and is most rapid where the forage is not grazed by cattle.

Laird (11), working with five important sod-forming grasses under pasture conditions, found that approximately 50 percent of the dry weight of the plants was in the root system and that most of the weight of the root was in the 8 inches just below the surface of the soil.

On the experimental area 30 species of native legumes,⁷ most of which are perennials, occur in considerable abundance. In 1931, after 9 years of burning, counts of the legume plants were made in each of the 0.01-acre plots in the four experimental areas. From these counts the number of legume plants per acre was calculated, as shown in table 3.

TABLE 3.—Comparison of legume plants on burned and unburned areas

Treatment of area	Legume plants per acre on—	
	Grazed area	Ungrazed area
Burned annually (9 years).....	Number 35,700	Number 41,500
Unburned.....	27,600	17,600
Difference.....	8,100	23,900

From tables 2 and 3 it appears that the cumulative smothering effect of unburned plant debris not only reduced the grasses on the protected areas but also reduced the legumes to the extent that after 9 years of fire protection they were less than half as numerous as on the area burned annually over the same period, where neither area had been grazed. These counts confirm observations made in Georgia by Stoddard (23) and in Florida by Harper (9). In the grazed areas the number of legumes on the protected plots also was materially less though the difference was not so marked.

A strip survey⁸ of the forage cover of the entire county in which the experimental area was located showed that the legumes were most abundant on the well-drained soils. This observation confirms those of previous investigators.

The burned and ungrazed area had one more legume plant for approximately each 2 square feet than had the unburned and ungrazed area. The probable effect of this difference in legume population on the quantity of nitrogen gathered from the air is obvious. It is also

⁷ The seeds of 20 species found at McNeill, Miss., have been determined by Stoddard (23) to be important quail feed.

⁸ A sampling process based on studies of representative small plots at regular intervals.

clear that doubling the plant growth per acre would greatly increase the accumulation of organic matter in the soil through decay of plant roots.

CRUDE PROTEIN AND ASH IN FORAGE

It is to be expected that the difference in nitrogen in soils from burned and unburned areas, as shown in table 1, would be reflected somewhat in a difference in crude protein in forage from those areas. The quantity of the protein in plants indicates the fertility of the soil, particularly soil that is deficient in nitrogen. To compare the forage from the burned and unburned areas for content of crude protein, and also for ash, samples of the two predominating species of grasses, *Andropogon scoparius* and *A. tener*, were taken in 1929 and again in 1931 after 7 and 9 years of burning and fire control, respectively. Composite samples were made by cutting and mixing the current growth from a large number of random locations. Samples were taken from April to June during the flush of the early season's growth. Table 4 presents a summary of the results obtained. No sample from the unburned areas equaled the comparable sample from the burned areas.

TABLE 4.—Comparison of the average crude protein and ash content of moisture-free samples of grasses on burned and unburned areas

Treatment of area	Crude protein	Ash
	Percent	Percent
Burned annually (7 and 9 years).....	10.15	7.92
Unburned.....	7.77	6.86
Difference.....	2.38	1.06

A difference of 2.38 percent in crude protein is sufficient to affect the feeding value of the forage, and the sale value of commercial feeds is determined largely by the difference in protein content. A difference of 1.06 percent in ash also would affect the value of forage plants as feed (3, 20).

MINERAL FERTILIZING ELEMENTS

In 1930, after 8 years of burning and fire control, soil samples representing the four soil types in the burned and unburned areas were analyzed for their content of mineral elements, namely, aluminum oxide, magnesium oxide, calcium oxide, potash, phosphoric acid, and sulphur trioxide. The total quantity of these minerals in the ash was as follows: Burned areas, average of all samples, 2.581 percent; unburned areas, 1.899 percent; difference in favor of burned areas, 0.682 percent. No sample from the unburned areas equaled the comparable sample from the burned areas.

These analyses of the mineral fertilizing elements of the soil substantiate the statements of previous investigators, already presented, that burning does not deplete the mineral fertility of the soil, but returns it directly to the soil where it becomes quickly available for the growth of plants.

SOIL MOISTURE

As a secondary influence of increased organic matter in the soil, it would be expected that soil moisture would increase because of the known water-holding capacity of organic matter in soil.

The region under consideration has a heavy rainfall distributed throughout every month of the year, November having the minimum amount. The average rainfall in November over a 27-year period was 3 inches. Ordinarily there is no deficiency in surface moisture except on steep slopes or light sandy soils, although during periods of drought the soil moisture is rapidly reduced.

The data obtained on soil moisture were based on samples representing different soil types and varying degrees of vegetative growth. Individual samples collected during the growing season accordingly showed considerable variation with no particular trend. In view of the rather wide variation in organic matter for different soil types and the patchy vegetative growth on the unburned pasture, due to spotted grazing, it was realized that the different conditions might give rise to unreliable comparisons unless large numbers of samples were taken. Accordingly more than a thousand individual samples were obtained to make more than 200 composite samples. These represented soil from the surface to a depth of 12 inches for both the burned and unburned areas.

Each of the composite samples was made up of from 5 to 10 individual samples taken within a radius of about 10 feet. No sample was taken less than 24 hours after a rainfall. The results of this investigation are given in table 5.

TABLE 5.—Average percentage of soil moisture in samples of soil from burned and unburned areas, Apr. 21 Oct. 9, 1931

Treatment of area	Composite samples	Moisture
	Number	Percent
Unburned..	124	9.63
Burned ..	119	9.46
Difference..	5	.17

Table 5 shows no significant difference in the moisture content of the burned and unburned areas. However, in considering the moisture data, the wide variation between plant growth on the burned and unburned areas must be taken into account since a ton crop of hay per acre requires the use of about 250 tons of water during the period of its growth (18). When the moisture taken from the soil and transpired by a much larger forage production is considered, the conclusion seems warranted that the additional organic matter in the soils on the burned areas must have increased their water-holding capacity to a considerable extent. This is further confirmed by moisture determinations of surface-soil samples taken in May 1930 during the flush of plant growth, and in December after the plant growth was mature. The popular conception is that a mulch of litter on top of the soil conserves moisture at the surface, and the samples which furnished the data shown in table 6 were taken to a depth of 1 inch.

TABLE 6.—*Soil moisture of burned and unburned areas at a depth of 1 inch*

Condition of area	Moisture content of soil ¹ on—		Condition of area	Moisture content of soil ¹ on—	
	May 21, 1930	Dec. 6, 1930		May 21, 1930	Dec. 6, 1930
Burned:	Percent	Percent	Unburned:	Percent	Percent
Grazed.....	10.3	11.1	Grazed.....	9.9	9.9
Ungrazed.....	9.3	14.9	Ungrazed.....	10.0	13.2

¹ Rainfall for the 30 days ended May 19 was 3.25 inches and for the 30 days ended Dec. 5, 6.37 inches. Of these quantities 2.09 inches fell on May 19 and 0.55 inch on Dec. 5.

Although the burned and ungrazed area produced a forage growth more than double that on the adjacent unburned and ungrazed area, it still maintained its moisture content and ended the growing season with 1.7 percent more moisture.

Merkle and Irvin (17) have shown that laboratory results in the conservation of moisture by a mulch, where the soil tubes are in contact with a water table, do not apply in practice to field conditions where no such water table exists near the surface of the soil. In the latter case, the conclusion seems warranted that the maintenance of soil moisture on the burned areas was due to the increased organic matter in the soil, and that the mulch of accumulated debris on the unburned area did not have the effect popularly described.

Water absorbed by plant debris, in place on top of the soil, is subject to the same sharp division from soil moisture that has already been made between organic matter on top of soil and that incorporated with the soil. It is obvious that any water absorbed by the litter is held away from the soil until it is evaporated. It is obvious also that evaporation is more rapid in the presence of free-air circulation than after moisture is absorbed in the soil. However, the water absorbed directly by the surface litter is a very small percentage of the total rainfall. Samples of a 9-year accumulation of three kinds of plant debris were carefully removed from measured areas on the experimental tract and air-dried. The quantity of water they would absorb to the saturation point was then determined by weight and calculated in terms of inches of rainfall. It was found that the maximum quantity, 0.11 inch, was absorbed by pine straw. Oak leaves absorbed 0.09 inch and dead grass 0.05 inch.

A mulch of plant debris in humid areas appears to affect the soil moisture chiefly by suppressing plant growth, which would take up moisture and transpire it through the leaves. The quantity of water absorbed by such litter is so small that it would not influence soil moisture to any appreciable extent, although it might absorb light showers and deprive surface-feeding plants of a temporary supply of moisture that would freshen plant growth in times of dry weather. Such effects on pastures from showers are well known. It has already been shown that accumulated litter did not raise the soil-moisture content even where it has suppressed plant growth about one-half. Any effect popularly ascribed to a leaf litter, in this case did not compensate for the increased soil moisture held in an adjacent soil with a higher organic-matter content, although the adjacent burned-over soil was supporting about twice the plant growth per acre.

SOIL FLORA

The micro-organisms of the soil are known to be greatly increased by the addition of organic matter, since the organic matter furnishes the energy and nitrogen necessary for growth and reproduction. However, counts of soil organisms vary greatly from time to time and are not a reliable index of soil fertility. The following counts are presented merely as being of interest.

The average bacterial count per gram of soil in 1930 was 1,242,000 for the burned and 857,000 for the unburned areas. In only 1 sample out of 11 did the count on an unburned sample exceed that on the corresponding burned sample. The difference in favor of the burned samples was 385,000 organisms per gram of soil. A larger number of bacteria in the burned soil would be expected in view of the greater amount of organic matter and nitrogen there, as shown by previous analyses. Samples also were collected before and after burning, for the same season. Of 5 sets of samples compared in this way, 3 showed decreases in soil organisms after burning and 2 showed increases. The increases were so great on the two samples, however, that the average increase following the fire was 258,000 organisms per gram of soil.

Coleman (5) has shown that the activities of soil organisms are greatest at temperatures between 86° and 100° F. The average of nine soil temperatures taken to a depth of 3 inches between March 16, 1924, and April 24, 1924, after a 3-year accumulation of plant debris on the unburned area, was 78° for the burned area and 72.5° for the unburned area, or a difference of 5.5° in favor of the burned area. It should be understood, of course, that the temperatures were not taken on or near the days of actual burning, and that the higher temperature was the nearer to the range of optimum temperatures. The results tend to account for the average higher bacterial counts following burning.

DISCUSSION

Although the soil and topography in southern Mississippi vary somewhat from those of other locations in the longleaf pine belt, the growth of grasses may be considered in general as typical for the entire area of well-drained lands. In a region more than 1,200 miles in extent, which once grew one species of tree in almost pure stands, it would be expected that factors influencing the growth of associated grasses and legumes on the forest floor would be nearly constant enough to produce in general a rather uniform herbage growth; and this growth is clearly associated with the effects of centuries of periodic grass fires.

To say that burning the organic matter in the form of plant debris on the forest floor or on top of virgin soils tends to increase the organic matter and nitrogen content of the soil may seem paradoxical. Yet it has been shown that the increase of organic matter in the soil is due primarily to the decay of plant roots and that incorporation of plant debris with the soil to form humus is an extremely slow process. It is evident also that any factor that increases the number of plants per acre will of necessity increase the formation of organic matter through the addition of roots to the soil, regardless of what becomes of the tops of the plants. In the experiments described the annual use of winter grass fires approximately doubled the growth of ungrazed grasses and legumes per acre over that produced on similar areas com-

pletely protected from fire, and caused a corresponding increase in the soil organic matter to a depth of 6 inches.

The soils considered are relatively heavy soils for the region. On lighter sandy soil, where the vegetative growth is much less dense, the reduction in plant growth through fire protection and the subsequent effect on the soil would necessarily be a much slower process.

It has been pointed out that nitrogen in the soil is derived from the gaseous form in the air, principally through the medium of bacteria associated with legumes, and that there is a constant loss of soil nitrogen which must be replaced if the soil nitrogen is to be held in balance or increased. In these experiments the annual use of winter grass fires maintained a legume growth on an ungrazed burned area about twice that on a similar area protected from fire, and the hypothesis seems entirely warranted that this increased legume population has caused a corresponding increase of nitrogen on the burned areas over that on the unburned areas. The difference shown by soil analyses may be due, however, partly to an increase of nitrogen on the burned areas and partly to a loss of nitrogen by leaching from the unburned soils where the current plant growth has been greatly reduced.

In studies of the effect of burning on mineral fertilizing elements of the soil, there was a marked difference in favor of the burned areas. Data obtained on soil moisture showed no significant differences, in actual moisture content, but the burned areas produced larger yields of plant growth with attendant larger moisture requirements which evidently were supplied. Data on soil micro-organisms indicate that burning tends to increase their number.

SUMMARY

Analyses of soils taken after 8 years of annual grass burning as compared with complete fire protection on rolling longleaf pine land in southern Mississippi showed 1.6 times as much organic matter in the burned-over soils as in the soils protected from fire. The burned-over soils also contained 1.5 times as much nitrogen as the soils protected from fire. The greater quantities of organic matter and nitrogen apparently result chiefly from roots rather than from tops of plants.

Whether plant debris was burned in place on top of the soil, or was left to rot in place on top of the soil, apparently had no direct effect on either the organic-matter content or the nitrogen content of the soil. In both cases, the organic matter and nitrogen aboveground were largely lost to the soil and the nonvolatile mineral fertilizing elements were returned, leaving organic matter and nitrogen increases to be influenced by the amount and composition of decaying plant roots.

Studies of grass and legume growth on the areas for periods of 8 and 9 years, respectively, showed that the quantity of forage growth on the ungrazed burned areas at the end of the period was more than double that on the unburned areas. The additional quantities of plant roots decaying in the soil on the burned areas apparently account for the increase in soil organic matter to a depth of 6 inches.

The increased growth, on the burned areas, of native legumes, their ability to take nitrogen from the air, and the additional growth of other plants which take up soluble forms of nitrogen and prevent leaching, apparently account for the increased amount of soil nitrogen.

The increase in organic matter and nitrogen on the burned areas was reflected in the higher crude-protein content of the principal forage grasses that grew on burned areas as contrasted with the unburned.

Annual burning returned the nonvolatile fertilizing elements to the soil immediately; this was shown in the analyses of both the soil and the forage growth.

The increased organic matter and nitrogen in the burned-over soils was reflected in an increased number of soil micro-organisms.

The accumulation of plant debris on top of the soil did not materially increase the soil moisture in spite of the fact that much greater amounts of water were required to support the extra forage growth on the burned-over soils.

Organic matter on top of the soil absorbs a portion of the rainfall which is thus prevented from reaching the soil for the use of growing plants.

On the forest floor or on virgin soils, that are not to be plowed, a sharp distinction must be made between the value of organic matter in the soil and organic matter in place on top of the soil.

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HISTOLOGICAL STUDIES OF WISCONSIN HOLLANDER AND WISCONSIN BALLHEAD CABBAGE IN RELATION TO RESISTANCE TO YELLOWS¹

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INTRODUCTION

The cabbage yellows organism, *Fusarium conglutinans* Wr., was reported as a vascular parasite by Smith (8)² and later by Harter (4), who showed that it formed microconidia in the vessels of the living plant. Tisdale (11) found that it penetrated the root hairs of cabbage plants growing on artificial media, and for some time this was accepted as its chief avenue of entrance of the host. Smith and Walker (9) found invasion to occur chiefly through the rootcap and the zone of elongation, and only infrequently through root hairs. Penetration was usually intercellular and the fungus made its way into the protosteles while that tissue was still undergoing differentiation. The avenue of entrance was the same in susceptible and homozygous resistant lines of cabbage, although penetration seldom occurred in the latter. Resistance to the fungus prevailed throughout the embryonic and permanent tissues of the root, and was attributed to antagonistic chemical substances or physiological qualities of the protoplasm, rather than to morphological characters of the tissues.

In the lines developed by Walker and his associates (12, 13, 14, 15, 16) resistance was shown to behave as a single dominant Mendelian factor. In a study of the breeding behavior of individuals of the Wisconsin Hollander variety of cabbage developed by Jones and associates (5, 6), Anderson (1) showed that resistance in that strain was not necessarily dominant nor was it controlled by a single factor. Phenotypically the resistance of Wisconsin Hollander was intermediate between that of the homozygous resistant lines and the susceptible commercial stocks in that it was commonly effective in the field but tended to break down under relatively high soil temperatures in the field or greenhouse.

The purpose of the present investigation was to study comparatively the host-parasite relationship in a susceptible Hollander strain of cabbage, in the intermediate resistant type of cabbage represented by Wisconsin Hollander, and in a homozygous resistant line developed from the same original commercial type, represented by the Wisconsin Ballhead variety already described by Walker and Blank (14).

MATERIALS AND METHODS

Wisconsin Hollander and Wisconsin Ballhead were both selected from the commercial variety known as Hollander or Danish Ballhead.

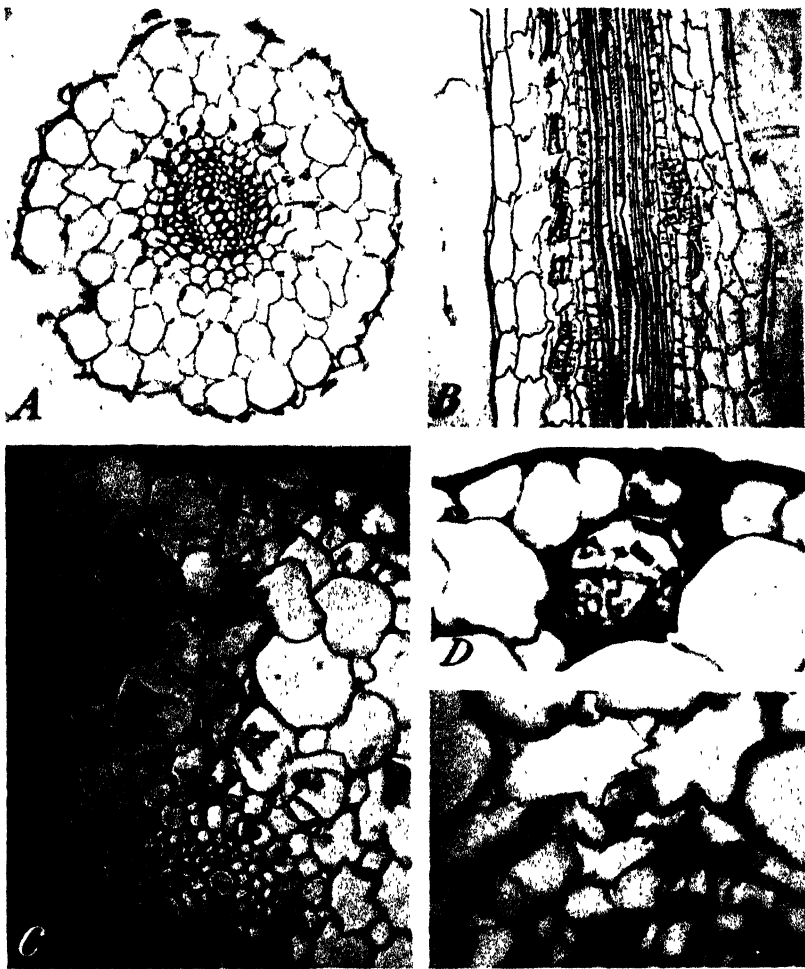
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² Reference is made by number (italic) to Literature Cited, p. 836.

The Wisconsin Ballhead seed used was lot no. 3331B, described by Walker and Blank (14). This was shown by their tests to be homozygous for resistance. Tests by Anderson (1) showed it to remain healthy in yellows-infested soil at constant soil temperatures as high as 26° C. Two single-plant progenies secured by Anderson (1) from Wisconsin Hollander were selected. One of these, progeny 33s, was very susceptible and will be referred to in this paper as susceptible Hollander; the other, progeny 11s, was one of the most resistant lines derived from this variety and is referred to hereafter as Wisconsin Hollander. It showed a high percentage of resistant plants in the field, but differed from Wisconsin Ballhead in that resistance of the parent plant was not completely dominant, while its progeny from self-pollination succumbed to yellows at soil temperatures of about 24° C.

In order to study different aspects of infection, plants were grown under three types of environment: (1) Seeds placed on artificial media inoculated with the yellows organism; (2) seeds planted directly in inoculated soil; and (3) 30-day-old plants transplanted into inoculated soil. For infection on artificial media, seeds were surface-sterilized in 1 to 1,000 mercuric chloride solution, washed in sterile water, and germinated on potato-dextrose agar. Soil-extract agar in Petri dishes was inoculated in several places across each plate with a single-spore culture of *Fusarium conglutinans*. Seeds in which the radicle was breaking through the seed coat were placed on the inoculated plates slightly in advance of the extending fungus. The culture plates were then placed in a constant temperature chamber at 24° C. and so arranged that the seedlings were held in a normal growing position. Seedlings grown directly in soil inoculated with a cornmeal-sand culture of the fungus were first germinated on potato-dextrose agar and then planted when the radicles were 2 to 3 mm in length. In this series two types of soil treatment were employed: (1) Virgin soil on which no cultivated crops had grown was inoculated directly; (2) greenhouse soil which was steamed under 10 pounds of pressure for 4 hours was later inoculated. The plantings were made in a greenhouse where the soil temperature ranged from 22° to 24°. In another series seedlings were grown for 30 days on soil free from the yellows organism and then transplanted to inoculated soil. The soil temperature was held quite constant at about 22° by the use of Wisconsin soil-temperature tanks.

At various intervals plants were removed for fixation and embedding in paraffin. Plants in soil were carefully removed and the surrounding soil loosened by gentle agitation in water. Even with utmost care plant parts were often missing, especially in severely infected roots. Formol acetic alcohol and Gilson's fixatives were employed, and the staining procedure followed throughout was a combination of Delafield's haematoxylin, safranin, and orange G. In most cases the whole root was cut in serial sections which were 10 to 15 microns in thickness. In the series where 30-day-old plants were transplanted to inoculated soil, sectioning was limited to the region just below the soil level where secondary roots were abundant.



WISCONSIN HOLLANDER ROOTS.

- A*, Two-day-old root showing suberized thickenings on the radial walls of the cortical cells surrounding the endodermis, $\times 136$; *B*, 3-day-old root showing the thickenings in the same layer of cortical cells, $\times 70$; *C*, 6-day-old root showing cortical infection extending inter- and intracellularly to the pericycle, $\times 190$; *D*, 6-day-old root grown on soil-extract agar, showing penetration of the epidermis and hypodermis accompanied by granulation of the cell contents, $\times 635$; *E*, root of a 6-day-old plant showing suberization of endodermis and pericycle successfully inhibiting fungus invasion of the xylem, $\times 444$.

NORMAL ANATOMY OF THE CABBAGE ROOT

Since the anatomy of the cabbage root has been discussed by earlier writers (7, 9, 17) only a brief description of the young root is given. The general structure of the young cabbage root with its diarch protosteles and alternate arrangement of xylem and phloem is shown in plate 1, *A* and *B*. A rootcap about six cells in thickness at the root tip decreases to a single layer of cells as it extends back along the sides of the root for a distance of about 1 mm. The epidermis gives rise to the root hairs beginning just above the zone of elongation.

The cortex consists of 4 to 5 layers of cells with large intercellular spaces. The endodermis is rather inconspicuous, but the layer of cells surrounding it is outstanding because of a heavily suberized thickening in each cell. This thickening forms an oval band or ring centrally located on the inner surface of the radial and cross walls. Smaller meshlike thickenings often extend from this main ring, especially centripetally, where they may extend over the inner tangential wall (pl. 1, *A* and *B*). This layer is supportive in nature and often persists when the outer part of the cortex is detached. These peculiar thickenings are not always present and may be totally lacking or interrupted in parts of the same root. Infrequently they occur locally on two rows of cortical cells. The cortical cells enlarge somewhat as root growth continues but soon become loosened and slough off. As the cortex becomes detached, a permanent periderm is formed as a result of tangential division of the pericycle cells. The characteristic root structure persists to the upper region of the hypocotyl.

Tims (10) and Smith and Walker (9) found no morphological differences in roots of susceptible and resistant plants. No morphological differences were noticed in the present study, but some differences in reaction to the parasite will be discussed later.

COMPARISON OF INFECTION IN SUSCEPTIBLE HOLLANDER, WISCONSIN HOLLANDER, AND WISCONSIN BALLHEAD

Plants of the susceptible and the two resistant strains were exposed to the fungus on agar plates and in inoculated soil. The roots and hypocotyls were fixed, embedded, and sectioned serially. In table 1 is a summary of the results secured from seedlings on agar plates. By the fifth day the root tips and the cortex and stele of both root and hypocotyl of susceptible Hollander were heavily infected. In Wisconsin Hollander (intermediate resistant) and Wisconsin Ballhead (homozygous resistant) invasion of the root tip and of the cortex of root and hypocotyl was common, while invasion of the stele was less pronounced.

TABLE 1.—Occurrence¹ of *Fusarium conglutinans* in the young roots and hypocotyl of susceptible Hollander, Wisconsin Ballhead, and Wisconsin Ballhead cabbage seedlings grown on agar plates at 24° C.

Period of exposure (days)	Susceptible Hollander						Wisconsin Hollander						Wisconsin Ballhead					
	Plant no.	Root			Hypocotyl		Plant no.	Root			Hypocotyl		Plant no.	Root			Hypocotyl	
		Root tip	Cortex	Stele	Cortex	Stele		Root tip	Cortex	Stele	Cortex	Stele		Root tip	Cortex	Stele	Cortex	Stele
2.	1	0	+	0	0	0	1	0	0	0	0	0	1	0	+	0	0	
	2	0	+	0	0	0	2	0	0	0	0	0	2	0	0	0	0	
	3	0	+	0	0	0	3	0	0	0	0	0	3	0	0	0	0	
	4	+	+	0	0	0	4	0	0	0	0	0	4	0	0	0	0	
	5						5	0	0	0	0	0	5	0	0	0	0	
	6						6	0	0	0	0	0						
	7						7	0	0	0	0	0						
	8	0	0	0	0	0	8	0	0	0	0	0						
	9	0	0	0	0	0	9	0	0	0	0	0						
	10	0	0	0	0	0	10	0	0	0	0	0						
	11	0	+	0	0	0												
	12	+	+	0	0	0												
	13	+	+	0	0	0												
3.	14	0	+	0	0	0	11	+	0	0	0	0	6	+	+	0	0	
	15	0	+	0	0	0	12	0	0	0	0	0	7	0	0	0	0	
	16	+	+	0	0	0	13	0	0	0	0	0	8	0	0	0	0	
	17	+	+	0	0	0	14	0	0	0	0	0	9	0	+	+	0	
	18	+	+	0	0	0	15	+	+	0	0	0						
							16	+	+	0	0	0						
							17	+	+	0	0	0						
							18	+	+	0	0	0						
							19	0	+	0	0	0						
							20	+	+	0	0	0						
							21	0	0	0	0	0						
							22	0	0	0	0	0						
							23	+	+	0	0	0						
5.	19	+	+	+	+	+	25						10	0	0	0	+	
	20	+	+	+	+	+	26	0	0	0	0	0	11	0	0	0	0	
	21	+	+	+	+	+	27	+	+	0	0	0	12	+	+	+	+	
	22	+	+	+	+	+	28	+	+	+	+	+	13	0	0	0	0	
	23	+	+	+	+	+	29	+	+	+	+	+						
	24	+	+	+	+	+	30	0	0	0	0	0						
	25	+	+	+	+	+	31	0	0	0	0	0						

¹ The symbols used in this table and in tables 2 and 3 denote the degree of infection as follows: 0=no infection; +=slight infection; ++=moderate infection; +++=severe infection.

In table 2 the results in unsterilized inoculated soil are given. After 6 days the susceptible plants were generally infected in cortex and stele. Most Wisconsin Hollander plants were infected in the root cortex and over half of them showed the fungus in the stele. In Wisconsin Ballhead, infection was general in the cortex but only an occasional plant showed stelar infection. Essentially similar results were secured when sterilized inoculated soil was used.

TABLE 2.—Occurrence¹ of *Fusarium conglutinans* in the young roots of susceptible Hollander, Wisconsin Hollander, and Wisconsin Ballhead cabbage plants grown from germinating seeds sown directly in unsterilized inoculated soil held at 22°–24° C. for various intervals

Interval (days)	Susceptible Hollander			Wisconsin Hollander			Wisconsin Ballhead		
	Plant no.	Degree of infection in—		Plant no.	Degree of infection in—		Plant no.	Degree of infection in—	
		Cortex	Stele		Cortex	Stele		Cortex	Stele
3	1	0	0	1	0	0	1	0	0
	2	+	0	2	0	0	2	0	0
	3	+	0	3	0	0	3	0	0
	4	+	+	4	+	+	4	+	0
6	5	++	+	5	++	0	5	++	+
	6	++	++	6	++	0	6	++	0
	7	++	++	7	++	0	7	++	0
	8	++	++	8	0	0	8	+	0
10				9	++	++	9	0	0
				10	++	+			
				11	+	0			
				12	+	0			
14	9	++	+	13	+	0	10	+	0
	10		+++	14		0	11	++	+
				15	++	+++	12	+	0
				16	++	++	13	++	0
18				17	++	++	14	+	0
				18	+	0	15	+	0
				19		++			
				20	+	0	16	+	0
				21	+	0	17	+	0
				22	++	+	18	0	0
							19	+	0

¹ For explanation of symbols see footnote to table 1.

In the examination of plants removed from the soil at various intervals there was a definite relationship between secondary root development and resistance. No secondary roots developed in the susceptible strain at 6, 10, and 14 days, and after the last period all were dead. The primary roots were brownish in color and had a collapsed appearance. It is to be seen in table 2 that at 6 days infection of this strain had occurred and the fungus had advanced into the stele. Undoubtedly it attacked the secondary roots before they emerged. In Wisconsin Hollander (intermediate resistant) plants at 6 days the primary roots were partially discolored and secondary roots were beginning to emerge. Root injury was more apparent as time went on, until at 18 days the only plants remaining turgid were those supported by secondary roots. At 6 days the primary roots of Wisconsin Ballhead (homozygous resistant) plants were normal in color and possessed well-developed secondary roots. Some injury to the primary root was apparent at 14 days and later but the secondary roots were normal in color and in turgidity.

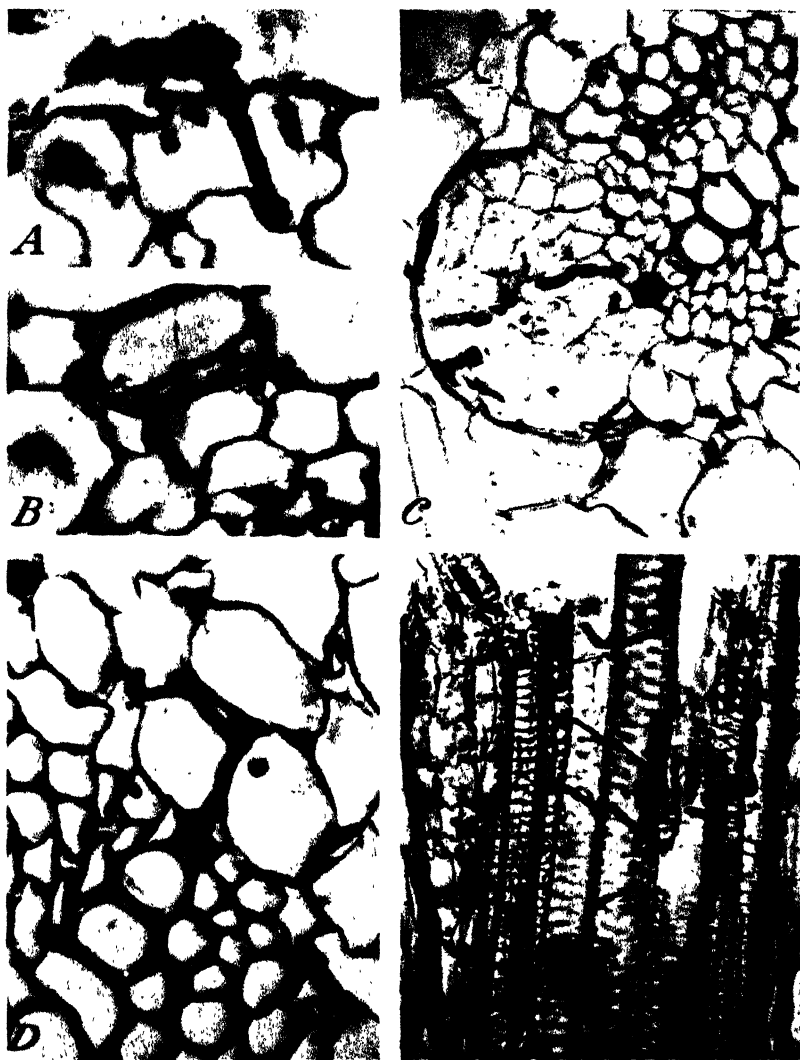
In table 3 are the results from 30-day-old seedlings transplanted to inoculated soil and grown at a soil temperature of 22° C. for various intervals. In these plants the roots were sectioned near the soil level where secondary roots were abundant. All plants of susceptible Hollander not previously sectioned were dead at the end of 17 days. Infrequent slight infection occurred in Wisconsin Hollander plants up to 14 days. At 17 days and longer moderate infection was common wherein a few vessels located in one section of the root were invaded. This type of localized infection of the xylem is in accord with the common field symptoms of this variety. In such cases unilateral disease development occurs or one or more lower leaves are affected and later drop off, leaving the plant without further outward sign of the disease (1). It is this type of infection which gives to Wisconsin Hollander the appearance of intermediate resistance or tolerance to the parasite. At high soil temperatures (24° C. or above) this resistance in Wisconsin Hollander is broken down and the fungus is no longer limited in its development. In this series infection was not found in the roots of homozygous resistant Wisconsin Ballhead.

TABLE 3.—Occurrence ¹ of *Fusarium conglutinans* in susceptible Hollander, Wisconsin Hollander, and Wisconsin Ballhead cabbage when 30-day-old seedlings were transplanted into infested soil and held at 22° C. soil temperature for various intervals

Interval (days)	Susceptible Hollander		Wisconsin Hollander		Wisconsin Ballhead	
	Plant no.	Degree of infection	Plant no.	Degree of infection	Plant no.	Degree of infection
7.....	1	+	1	0	1	0
	2	+++	2	0	2	0
	3	+++	3	+		
	4	+++	4	0		
9.....	5	+++	5	0		
	6	+++	6	0		
	7	+++	7	0	3	0
11.....	8	+++	8	0	4	0
	9	+++	9	0	5	0
			10	+		
14.....	10	+++	11	0	6	0
	11	+++	12	0	7	0
			13	0	8	0
	12	+++	14	0	9	0
17.....			15	+	10	0
			16	++	11	0
			17	++	12	0
20.....			18	++	13	0
			19	0	14	0
24.....			20	++	15	0

¹ For explanation of symbols see footnote to table 1.

It is evident from this comparative study that prompt infection of cortex and stele of susceptible Hollander plants occurs. The infection of Wisconsin Ballhead roots is usually found to be limited to the epidermis and cortex of roots and hypocotyl. Seldom does the fungus increase in the cortex to the same degree that it does in susceptible plants. Such invasions apparently cause little damage and disappear with the sloughing of the cortex. Occasionally the fungus was found to have reached the stele in this variety but, with one exception, infection in the stele was very slight. In the one exceptional case the root tip was severely attacked and the fungus advanced a short distance into the stele. Cortical infection was generally more



SUSCEPTIBLE HOLLANDER ROOTS.

- A*, Penetration of the root epidermis in which there is an enlargement of the fungus hypha at the surface and invasion of the cell wall by a peglike constriction, $\times 1015$; *B*, intracellular passage of cortical layer surrounding endodermis, the suberized thickening being shown in the wall of the infected cell, $\times 571$; *C*, 10-day-old root showing infection of a young secondary root by hyphae invading from the surrounding cortex, $\times 365$; *D*, 10-day-old root, showing a hypha crossing a cell of the pericycle and penetrating a protoxylem vessel, $\times 1015$; *E*, longitudinal section through the stele of the root of a 10-day-old plant showing passage of the fungus through the thin-walled parts of spiral vessels and through pits of reticulate vessels, $\times 412$.

severe in Wisconsin Hollander than in Wisconsin Ballhead. Infection of the stele was more frequent and the fungus advanced higher in the root. Thus this strain showed an intermediate degree of susceptibility or resistance to invasion which corresponds to the intermediate resistance commonly observed in the field.

PATHOLOGICAL ANATOMY OF THE CABBAGE ROOT

PENETRATION

Smith and Walker (9) studied penetration in plants grown on artificial media. They reported penetration chiefly in the embryonic region of the root, at times through root injuries, and infrequently by way of root hairs. The method of entrance was found to be intercellular and infrequently intracellular. In the present study no essential difference in penetration or subsequent development of the fungus was noticed between plants grown on soil-extract agar and those grown in inoculated soil. Penetration occurred within a period of 2 days in susceptible plants grown on agar at 24° C. and within 3 days in plants grown in inoculated soil at 22° (table 1). In the soil cultures infection occurred earlier and in a greater percentage of susceptible plants than in either of the resistant strains (tables 2 and 3). No difference in the process of penetration was noticed in the three strains of cabbage.

Infection of root hairs (fig. 1, A) is seldom found and is probably of no importance as an avenue of entrance to the host. Penetration occurs often in the root tip of susceptible plants, especially by way of the rootcap. The fungus often masses between and in the loose cells of the rootcap and continues upward to infect the growing tip. Often the growing point is not attacked and the infected root cap cells remain along the sides of the young root as the tip continues its growth.

Penetration is not limited to the young embryonic root structures but also occurs in the epidermis and outer cortical cells of the young root and hypocotyl. Through this avenue of entrance infection often occurs while the root tips remain free from infection (table 1). Infrequently the young root escapes the fungus and infection occurs only in the hypocotyl region. Sometimes infection through the hypocotyl in 30-day-old transplants was noticed in susceptible Hollander. The fungus also may gain entrance where secondary roots break through the cortex (pl. 2, C').

Direct intercellular and intracellular penetration (fig. 1, A to D; fig. 2, B, D) occur equally often, both in the young root and in the hypocotyl, while in the rootcap penetration is generally intercellular. In penetrating the epidermis the fungus generally neither shows change in thickness nor becomes distorted as if exerting pressure. Occasionally a cell wall is distended (fig. 1, B) as if from pressure. Infrequently a strand becomes thickened at the cell surface and penetrates by a smaller projection through the cell wall (pl. 2, A).

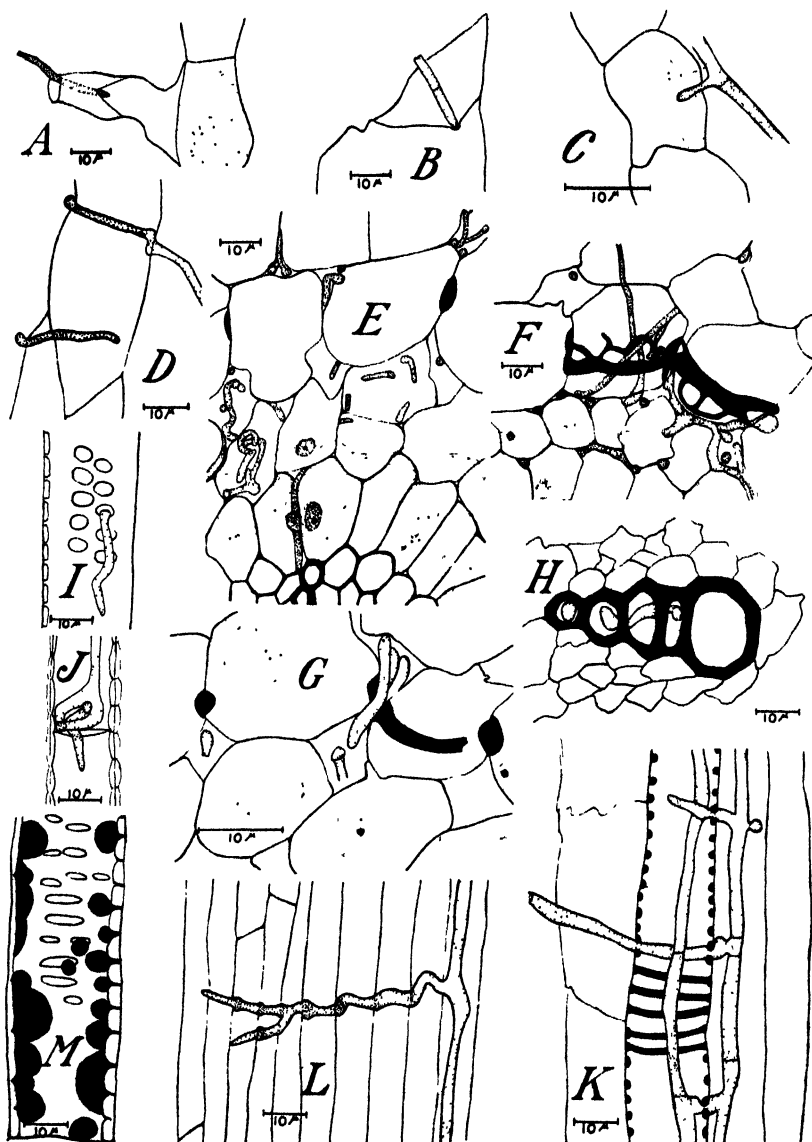


FIGURE 1.—Penetration and development of *Fusarium conglutinans* in cabbage roots: A, Fungus entering through root hair; B, penetration in which the inner cell wall is distended, an infrequent occurrence; C, D, direct penetration through epidermis; E, fungus in inner cortex and pericycle; F, cortical cells with suberized thickenings next to the endodermis being traversed intracellularly; G, an infrequent occurrence where the fungus forces passage between two cells of the layer surrounding the endodermis (note that the suberized thickenings do not meet in these two cells); H, fungus passing laterally through a vessel by way of border pits; I-K, fungus in vessels, entrance having been effected through the thin border pits by a conforming constriction of the hyphae; L, fungus passing laterally through several cells of the phloem, with bending of the hypha and enlargement near the cell wall as if due to stress and resistance in penetrating walls of this tissue; M, orientation and formation of occlusions in a vessel. (All drawings are from susceptible Hollander plants with the exception of D, which is from Wisconsin Hollander.)

SUBSEQUENT COURSE OF INVASION
INVASION THROUGH THE CORTEX

In the embryonic roots of susceptible plants, as reported by Smith and Walker (9), the fungus readily makes its way to the vascular system. The embryonic growing point is easily attacked and the root is sometimes stunted, especially when infection by the fungus occurs en masse through the rootcap. More often, however, infection takes place through the epidermis and cortex of the root (table 1).

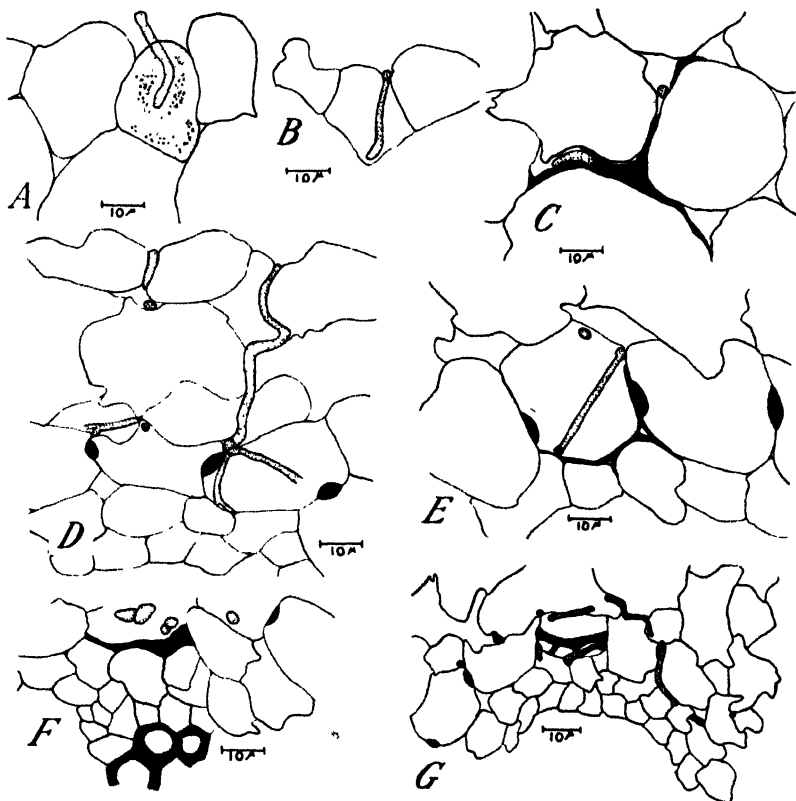


FIGURE 2.—Penetration and development of *Fusarium conglutinans* in homozygous resistant Wisconsin Ballhead cabbage: A, Penetration of epidermal cell accompanied by granulation of the cell contents; B, direct penetration of epidermal cell; C, deposition of gumlike material between cortical cells in advance of the fungus, an infrequent reaction not effective in preventing deeper invasion into the cortex; D, Fungal strand extending through cortex to endodermis, penetration being intercellular but the layer of cells surrounding the endodermis is crossed intracellularly; E, suberization of walls between endodermis and the surrounding layer of cortical cells; F, suberization between endodermis and pericycle, effectively barring the fungus from the stele; G, fungus in the cortex of a resistant root, the layer surrounding the endodermis being traversed intracellularly where radial suberized thickenings naturally occur, intercellular passage being likely to occur where these thickenings are lacking.

When epidermal calls are penetrated directly, the fungus soon becomes intercellular. It generally remains intercellular in its course across the cortex, although it sometimes occurs within and passes through cortical cells (fig. 1, E; pl. 1, C). The rigid suberized inner bands in the cells of the cortical layer surrounding the endodermis, with but few exceptions, inhibit intercellular passage. Intracellular penetration of this layer takes place readily, however, in old and young roots and in both susceptible and resistant plants when infec-

tion of the latter occurs (fig. 1, *F*; fig. 2, *D*; pl. 2, *B*). In one case (fig. 1, *G*) the fungus forced an intercellular passage, but in that case the thickenings on the adjacent cell walls did not make a good parallel fit. As stated above, these thickenings may be lacking or partly lacking, and in such cases intercellular penetration occurs (fig. 2, *G*). Thus, although it possesses suberized thickenings, this layer was not a barrier to radial ingress of the fungus in the cabbage varieties studied, as found by Smith and Walker (9) in other varieties.

Although *Fusarium congenitans* is primarily a vascular parasite, it is often found in the cortex for a considerable distance in the root and hypocotyl, as a result of repeated infection and of vertical advance of the fungus. The large intercellular spaces make convenient runways for the hyphae. When repeated infection does not occur, the fungus advances highest in the cortex in the intercellular spaces just outside the endodermal layer. Sometimes the fungus advances higher in the cortex than in the stele. This was especially noticeable in the two resistant strains, and may be due to incompatibility of fungus and host in the vessels.

The fungus in the cortex is an important source of secondary infection. Secondary roots are frequently attacked before they completely traverse the cortex of the primary root (pl. 2, *C*). In several cases initial infection of the stele of the main root resulted from such invasion of secondary roots. The cortex of the primary root infrequently appears predisposed to initial penetration as the result of outward growth of secondary roots since the fungus sometimes invades this region as the secondary root is about to push through. It occasionally enters the cortex of the primary root after the latter has been ruptured by the secondary root.

DEVELOPMENT IN THE STELE

The fungus apparently encounters more difficulty in passing the endodermis and pericycle than the cortical layer surrounding the endodermis. These tissues are usually traversed intracellularly. Generally the protoxylem vessels are next attacked, the fungus passing through the thin vessel wall between spiral thickenings. Plate 2, *D*, shows the passage of a strand through the pericycle into the protoxylem vessel. The vessels centripetally become reticulate and pitted and the fungus passes from one vessel to another through pits (fig. 1, *H-K*; pl. 2, *E*). The fungus seems to have an affinity for the protoxylem vessels, from which it repeatedly passes into the larger reticulate vessels.

The phloem and parenchymatous cells of the stele are also invaded by the fungus. In the phloem the fungal strands often appear much larger in cross section than in the xylem, apparently the result of the turning and enlargement of the strands in the process of penetration of cell walls. The crooked course of fungal strands with enlargement before wall penetration suggests that the fungus encounters more mechanical resistance in the phloem tissue. This phenomenon is noticed in both susceptible and resistant roots. Figure 1, *L*, shows a strand passing through several cells of the phloem.

REACTION OF HOST TO PARASITE

Although no structural differences in the three strains of cabbage were noticed, there were differences in host reaction in the presence of the fungus.

PROTOPLASMIC REACTION

Frequently, when penetrated directly, the protoplasmic content of the cells became granular and stained deeply with Delafield's haematoxylin. The cells appeared to be killed, and often ruptured. Under such conditions the fungus sometimes became granular and showed various stages of disintegration. Often, however, it passed through such cells into the intercellular spaces, where it continued to grow with no subsequent deleterious reaction of host cells or parasite.

This host reaction, while not extremely common, was more frequent and more pronounced in plants growing on artificial media. It was more often observed in both resistant varieties than in the susceptible strain. Figure 2, *A*, shows penetration of an epidermal cell in a Wisconsin Ballhead plant followed by protoplasmic granulation. Plate 1, *D*, shows a similar reaction in Wisconsin Hollander.

This reaction is suggestive of that found in cereals resistant to rust, although in the latter case cells and fungus succumb as a result of incompatibility. The reaction in cabbage, although not of great importance since it neither occurs uniformly nor is effective in checking the fungus, is interesting in that it is more pronounced in resistant than in susceptible plants.

CELL-WALL REACTION

Occasionally the walls of cortical cells of Wisconsin Ballhead appeared to become suberized slightly in advance of the fungus, as indicated by their staining red with safranin (fig. 2, *C* and *E*). A similar reaction was more common in the endodermis and pericycle of both resistant strains and less so in susceptible plants. Figure 2, *F*, shows the wall between endodermis and pericycle of a Wisconsin Ballhead root heavily suberized. In plate 1, *E*, the inward advance of the fungus was checked by endodermal and pericyclic suberization in a Wisconsin Hollander plant. This localized suberization in plants of the two resistant strains was especially noticeable where the infected cortex was in the process of detachment, and appeared to be effective in limiting the ingress of the fungus into the stele.

Too much emphasis should not be placed on these forms of host reaction, since they do not occur uniformly in all plants. At times, however, they do apparently play an important role in excluding the fungus from the stele. The real seat of resistance must be found in the nature of the cell contents which are apparently not favorable to a vigorous invasion and development by the fungus in intermediate resistant and in homozygous resistant plants.

OCCLUSIONS

Deposits of gumlike material in xylem vessels and parenchyma of cabbage were discussed by Smith and Walker (9). Occlusions were noticed by them in both susceptible and resistant roots growing in soil, but only in resistant roots growing on artificial media. In the present study infrequent occlusions were found in all strains, in both soil cultures and plants grown on artificial media. Occluded vessels were found most often in the series in which germinating seedlings were placed directly in inoculated soil. These seedlings after 10 to 18 days exhibited various degrees of injury in which cortex and even parts of the stele were lacking in young roots. Throughout the study occlu-

sions were seldom observed in the same vessels with fungal strands but were more often found in adjacent vessels. They also occurred where no trace of the fungus could be found. Their formation is shown in figure 1, *M*. Droplets of a gumlike substance apparently have their beginning at the pits of the vessel and may increase until they completely plug the latter. Generally but few vessels of an infected root show these gumlike deposits.

DISCUSSION

In this investigation a study of the intimate relation of the yellows organism to susceptible and resistant hosts has been undertaken. Stable resistance, controlled by a single dominant gene (Wisconsin Ballhead), was compared with a definite but more variable type not completely dominant and controlled apparently by a number of factors which merely modify the expression of the gene for susceptibility (Wisconsin Hollander).

In an earlier paper Smith and Walker (9) reported a similar study of susceptible lines and homozygous resistant lines of different origin. They were unable to determine any morphological basis for resistance. Tisdale (11), working with flax wilt (*Fusarium lini* Bolley), noted in the resistant strains a tendency of the cell walls of the cortex to thicken and show signs of suberization in advance of the fungus. He regarded this as a partial explanation of resistance but believed that the interaction between hyphae and the host protoplasm was perhaps more important in checking the parasite. Fahmy (3) in a study of cotton wilt (*F. vasinfectum* Atk.) described penetration through the root cap, while Dharmarajulu (2) considered resistance to be based upon a combination of cell-wall reaction and antagonism of the protoplast.

The writers have found that penetration in susceptible Hollander cabbage and in the two resistant strains proceeds in essentially the same manner. In all of these strains there appears to be more penetration of the cortex of root and hypocotyl than was found in other strains of cabbage by Smith and Walker (9). Moreover the cell-wall thickenings of the inner cortical layer of the root do not obstruct fungus ingress into the stele since the parasite penetrates these cells intracellularly.

While under the conditions of these studies there was invasion of the cortex of a larger percentage of resistant roots than had been reported for the lines studied earlier (9), invasion of the stele in the homozygous resistant Wisconsin Ballhead was rare and meager in extent. The parasite was obviously retarded in the cortex. This inhibition of growth was not correlated with any consistent morphological or biochemical reaction which could be demonstrated by histological methods although cell-wall suberization and granulation of the protoplasm occasionally occurred. These reactions, it is true, were somewhat more frequent in the resistant lines than in the susceptible line, but they were nevertheless too infrequent to account for the marked and distinct resistance which occurs in Wisconsin Ballhead.

At the outset of this study it was thought that since the resistance in Wisconsin Hollander had been shown to be distinct in its genetical basis from that of Wisconsin Ballhead, it might show a distinct histological picture. The results presented herein, however, indicate that the intermediate type of resistance exhibited by this strain differs in

degree rather than in kind from that of the homozygous resistant line. The relation of the parasite to this host is of the same order. There are, again, no consistent morphological reactions which can be interpreted as the basic cause of resistance. The fungus is more active in the cortex and is not so consistently excluded from the stele as is the case with Wisconsin Ballhead. Whether or not the same basic factors, in a lesser degree, as those which occur in Wisconsin Ballhead are responsible has not been demonstrated. It is clear, however, that whatever the nature of resistance may be in either resistant strain, it is not readily discerned by histological methods. It may be due to antagonistic compounds in the host cell or it may be simply an inherent inability on the part of the parasite to live and thrive in the host cell as successfully as it does in that of the susceptible strains. It would appear that further knowledge of the nature of resistance to *Fusarium conglutinans* can be secured best through a biochemical approach to the question.

SUMMARY

A comparative study was made of the invasion of three strains of Hollander cabbage by the yellows organism (*Fusarium conglutinans* Wr.): (1) a very susceptible strain of Hollander, (2) an intermediate resistant type (Wisconsin Hollander), and (3) a homozygous resistant strain (Wisconsin Ballhead).

The fungus enters the three strains of cabbage through the root tip and through the cortex of the young root and hypocotyl. Penetration of cell walls is apparently accomplished by mechanical pressure.

The fungus traverses the cortex of susceptible roots readily and often persists in this tissue as high as the upper hypocotyl. Secondary roots are often attacked by the pathogen as they push through the cortical tissue.

Penetration of Wisconsin Ballhead was quite frequent but the fungus was generally limited to the outer cortical cells or the lower root-tip region and very seldom reached the vascular system.

In Wisconsin Hollander the pathogen was retarded but not to the same extent as in Wisconsin Ballhead. Infection of the vascular system occurred more often but here the fungus was usually restricted to a few localized vessels.

No definite morphological differences occur in the strains of cabbage studied; however some differences in reaction of the host to the fungus were apparent in the resistant strains.

Only in Wisconsin Ballhead was intercellular suberization of cortical cells in advance of the fungus noted. Suberization of endodermis and pericycle walls often occurred locally in both Wisconsin Ballhead and Wisconsin Hollander when these tissues were approached by the fungus. Granulation of epidermal and outer cortical cells was noticeable in certain plants of Wisconsin Ballhead and Wisconsin Hollander when these cells were penetrated directly.

The basis of resistance in Wisconsin Hollander appears to differ in degree rather than in kind from that of Wisconsin Ballhead. Whatever the true nature of resistance may be, it is apparently not readily discernible by histological methods, and further knowledge regarding it may best be sought through a biochemical approach to the question.

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NUTRIENT VALUE OF THE PHOSPHORUS IN CALCINED PHOSPHATE AS DETERMINED BY GROWTH OF PLANTS IN GREENHOUSE EXPERIMENTS¹

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INTRODUCTION

The complex minerals fluorapatite ($\text{Ca}_{10}\text{F}_2(\text{PO}_4)^6$) and hydroxy-fluorapatite ($\text{Ca}_{10}(\text{OH}, \text{F})(\text{PO}_4)^6$) are the principal phosphatic constituents of nearly all commercial varieties of phosphate rock mined throughout the world (11, 13).²

All commercial varieties and grades of domestic phosphate rock contain about 3 to 4 percent of fluorine (13), and the percentage of fluorine in the rock is invariably higher than that corresponding to the fluorapatite equivalent of the total phosphorus content. The state of combination of the excess fluorine is not definitely known, but the available evidence indicates that it is probably present chiefly as calcium fluoride. If it is assumed that all the phosphorus in domestic phosphate rock is present in the form of fluorapatite, the fluorine in excess of that corresponding to the fluorapatite equivalent of the total phosphorus amounts to approximately 10 to 30 percent of the total fluorine. Therefore, about 70 to 90 percent of the fluorine is equally distributed between the two atoms of fluorine in the fluorapatite; for convenience these may be designated as the first (F_a) and second (F'_a) atoms of fluorine. Finally, the fluorine in excess of that corresponding to the second (F'_a) atom of fluorine in the fluorapatite equivalent of the phosphorus amounts to about 55 to 65 percent of the total fluorine.

Recent laboratory studies (17, 22, 23) have shown that when domestic phosphate rock, containing about 5 to 10 percent of silica, is heated in the presence of water vapor for 30 minutes at 1,375° to 1,425° C. it is possible to volatilize upward of 95 percent of the total fluorine (corresponding to upward of 86 percent of the F'_a fluorine) and convert 80 percent or more of the phosphorus into the citrate-soluble (available) condition. In order to obtain efficient reaction in the minimum time, the process must be regulated to give the maximum degree of contact between the water vapor and the particles of phosphate rock.

In experiments (22) with Florida land-pebble phosphate, containing 3.67 to 3.85 percent of fluorine and 6.80 to 10.85 percent of silica,

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² Reference is made by number (italic) to Literature Cited, p. 847.

volatilization of less than about 65 percent of the total fluorine (corresponding approximately to the fluorine in excess of the $F'_{\frac{1}{2}}$ fluorine) was accompanied by a decrease in the citrate solubility of the phosphorus in the product, as compared with that of the phosphorus in the original rock. From that point, however, the citrate solubility of the phosphorus corresponded approximately to the percentage of the $F'_{\frac{1}{2}}$ fluorine volatilized, provided the temperature of the experiment exceeded $1,300^{\circ}\text{C}$. The defluorinated material (calcined phosphate) contains little or no water-soluble phosphorus. The chemical nature of the citrate-soluble phosphorus has not been definitely determined, but it is believed to consist principally of the high-temperature form of tricalcium phosphate (3, 26).

Various processes have been proposed for the manufacture of available phosphates by heating phosphate rock with alkali salts, usually the sulphates or carbonates of sodium and potassium. These processes have been discussed by Fishburne (6), Guernsey and Yee (8), and Waggaman and Easterwood (27). As yet, attempts to operate such processes on a commercial scale have not met with sustained success in this country. In Germany, however, a product known as Rhenania phosphate (18, 24) has been manufactured for some years by heating phosphate rock with alkali salts under certain conditions. In this country Allison, Braham, and McMurtrey (1), Conner (4), Haskins (9, 10), and Mooers (19), and, in Europe, Erdély (5), Gerlach and Nolte (7), Jakobsen (16), Niklas, Strobel, and Scharrer (20), and R  th (21) have shown that citrate-soluble phosphates, prepared by heating phosphate rock with alkali salts, are excellent sources of phosphorus for plant growth.

Inasmuch as the process which involves the heating of silica-bearing phosphate rock in the presence of water vapor (without the addition of alkali salts) seems to have commercial possibilities, greenhouse pot experiments were carried out on four types of soil in order to obtain information on the nutrient value of calcined phosphates in which the phosphorus showed a wide range of citrate solubilities. The Alabama, Arkansas, and West Virginia Agricultural Experiment Stations and the Bureau of Plant Industry, United States Department of Agriculture, cooperated in the greenhouse experiments. The results are presented in this paper.

MATERIALS AND EXPERIMENTAL METHODS

Data on the preparation and composition of the phosphates are given in table 1.

Samples 3 and 4 were commercial phosphate rock from a deposit of unknown location in the Florida land-pebble phosphate district. The colloidal phosphate (sample 5) was a natural claylike material (12, 13) from an abandoned phosphate-washer waste pond in the Florida hard-rock phosphate district. The Tennessee brown-rock phosphate (sample 6) was a commercial kiln-dried material from a deposit near Mountpleasant, Tenn.

Samples 7, 8, 9, and 10 (calcined phosphates) were prepared in a laboratory tube furnace by heating Florida land-pebble phosphate rock, of the general composition represented by samples 3 and 4, in the presence of water vapor.³ In this rock, the fluorine in excess

³ These samples were prepared by D. S. Reynolds, Fertilizer Investigations, Bureau of Chemistry and Soils.

of that corresponding to the second (F'_B) atom of fluorine in the fluorapatite equivalent of the total phosphorus, amounted to approximately 63 percent of the total fluorine. In confirmation of previous results (22), no increase in the citrate solubility of the phosphorus was obtained until all the fluorine in excess of that corresponding to the second (F'_B) atom of fluorine in the fluorapatite equivalent of the total phosphorus was volatilized. Volatilization of only 50 percent of the total fluorine was accompanied by a decrease in the citrate solubility of the phosphorus in the product (sample 7), as compared with that of the phosphorus in the original rock.

TABLE 1.—*Phosphate materials used in greenhouse experiments*

Sample no.	Phosphate material	P_2O_5		Availability of P_2O_5	Total fluorine content	Total fluorine volatilized	F'_B fluorine ¹ volatilized
		Total	Available ²				
1	Florida pebble superphosphate, 20-mesh	Percent 20.40	Percent 20.32	Percent 99.6	Percent 1.65	Percent 31	-----
2	Dicalcium phosphate, $CaH_2PO_4 \cdot 2H_2O$, c. p. material, 80-mesh	41.52	41.52	100.0	0.00	-----	-----
3	Florida pebble phosphate, untreated material, 200-mesh	31.25	4.12	13.2	3.85	-----	-----
4	Florida pebble phosphate, same as no. 3 except 100-mesh	31.39	3.64	11.6	3.85	-----	-----
5	Colloidal phosphate, 100-mesh	23.03	2.39	10.4	1.92	-----	-----
6	Tennessee brown-rock phosphate, untreated material, 100-mesh	33.77	2.58	7.6	3.67	-----	-----
7	Calcined phosphate; 10-g charge of 200-mesh Florida pebble heated 0.5 hour at 1,300° C	33.88	2.30	6.8	2.08	49.9	0.0
8	Calcined phosphate; 10-g charge of 200-mesh Florida pebble heated 0.5 hour at 1,400° C	34.09	7.01	20.6	1.49	64.2	1.6
9	Calcined phosphate; 10-g charge of 40- to 80-mesh Florida pebble heated 1.0 hour at 1,400° C	33.38	16.39	49.1	.85	78.9	44.0
10	Calcined phosphate; 5-g charge of 40- to 80-mesh Florida pebble heated 1.0 hour at 1,400° C	33.78	26.39	78.1	.27	93.3	82.2
11	Calcined phosphate, prepared from Tennessee brown-rock phosphate	33.97	22.21	65.4	.40	90.0	76.0
12	do	34.21	29.46	86.1	.10	97.0	93.0
13	do	35.09	32.52	92.7	.10	97.0	93.0

¹ Neutral ammonium citrate method.

² Fluorine corresponding to the second atom of fluorine in the fluorapatite equivalent of the total phosphorus.

³ Contained 17.5 percent of water-soluble P_2O_5 .

⁴ Approximate figure.

⁵ Ground to 100-mesh.

⁶ Ground to 80-mesh.

Samples 11, 12, and 13 (calcined phosphates) were prepared in an experimental direct-fired rotary kiln from a commercial grade of Tennessee brown-rock phosphate.⁴

The conditions of the greenhouse experiments are outlined in table 2. In all experiments the potash and phosphate fertilizers were uniformly mixed with the entire mass of soil just before the seed was sown or the plants (cabbage) were transplanted; the initial applications of nitrogen fertilizers were made in the same manner. For a given series of experiments, the phosphates were applied on the basis of equal quantities of total phosphoric oxide (P_2O_5). In the second series of experiments by the Department of Agriculture chemically pure calcium carbonate, at the rate of 2,000 pounds per acre, was applied with the nitrogen, phosphate, and potash fertilizers to one set of pots; in contrast to the first and third series, the plants in this series were harvested before the seed heads were formed.

⁴ These samples were supplied by Paul Caldwell, Moorman Manufacturing Co., Quincy, Ill.

TABLE 2.—Conditions of greenhouse experiments

Item	Alabama Experiment Station	Arkansas Experiment Station	U. S. Department of Agriculture	West Virginia Experiment Station
Replications	2	3	3	3
Soil type and pH	Cecil clay, pH 6.0.	Clarksville silt loam, pH 6.23.	Norfolk loamy fine sand, pH 4.8.	Dekalb silt loam, pH 5.25.
Weight of air-dry soil per pot	8 l.	10 l.	5 l.	7 l.
P ₂ O ₅ application ¹	0.64 kilograms	0.334 kilograms	0.6 kilograms	0.35 kilograms
P ₂ O ₅ application ²	160 grams per pot	66.8 grams per pot	240 grams per pot	100 grams per pot
Nitrogen fertilizer	NaN ₃ O ₃ , 600 ¹	(NH ₄) ₂ SO ₄ , 300.	NaN ₃ O ₃ , 260; (NH ₄) ₂ SO ₄ , 195.	Urea, 200; NaN ₃ O ₃ , 500. ²
Potash fertilizer	KCl, 100.	KCl, 300.	Manure salts (27.5 percent K ₂ O), 436.	KCl, 100.
Crop	Cabbage	Sudan grass.	Millet.	Sudan grass.
Date of seeding	Feb. 28, 1934.	June 21, 1934.	Jan. 25, 1934.	May 7, 1934.
Plants per pot	4.	12.	10.	6.
Date of harvesting	Apr. 24, 1934.	Aug. 8, 1934.	Apr. 3, 1934. ²	Aug. 7, 1934.

12-gallon pots.

7-gallon pots.

Total P₂O₅.

300 pounds as top dressing and 300 pounds as top dressing.

200 pounds at seeding and 100 pounds as top dressing when plants were about half grown.

Applied as top dressing on Oct. 2, 1934.

Date of transplanting.

First series; second and third series planted July 21 and Aug. 1, 1934, respectively.

First series; second and third series harvested Aug. 28 and Sept. 24, 1934, respectively.

EFFECT OF PHOSPHATES ON OVEN-DRY WEIGHT AND PHOSPHORUS CONTENT OF PLANTS

The average oven-dry weight and phosphorus content of the plants are given in table 3.⁵ All of the soils were deficient in available phosphorus, and marked increases in the growth and phosphorus content of the plants were invariably obtained by the application of either superphosphate, dicalcium phosphate, or certain of the calcined phosphates. For a given series of experiments, the effect of the phosphates on the phosphorus content of the plants was, with a few exceptions, in the same order as their effect on the dry weight of the plants.

At the Alabama station, the 200-mesh Florida pebble phosphate rock was an excellent source of phosphorus for cabbage on Cecil clay of pH 6.0. At the West Virginia station, this material was also an excellent source of phosphorus for millet on Dekalb silt loam of pH 5.25, whereas it gave very poor results with Sudan grass on Dekalb silt loam of pH 6.10. Furthermore, the ground phosphate rock was not an efficient source of phosphorus for millet on Norfolk loamy fine sand of pH 4.8 (U. S. Department of Agriculture) and for Sudan grass on Clarksville silt loam of pH 6.23 (Arkansas station). Under comparable conditions, the 200-mesh Florida pebble phosphate appeared to be somewhat more efficient than the 100-mesh material. The Florida pebble phosphate gave better results than did either the colloidal phosphate or the Tennessee brown-rock phosphate. Under the conditions of these experiments, the nutrient value of the ground phosphate rock was not correlated with either the type or pH of the soil or the type of crop grown.

When the phosphate rock was calcined in such a manner as to volatilize only 50 percent of the fluorine the product (sample 7) was invariably less effective than the untreated rock in promoting plant growth and absorption of phosphorus. Volatilization of larger percentages of the fluorine usually had a marked progressive effect in increasing the nutrient value of the phosphorus (samples 8 to 13), the effects on plant growth and absorption of phosphorus being related, more or less directly, to the amount of fluorine volatilized and to the corresponding citrate solubility of the phosphorus. In general, calcined phosphates in which the availability of the phosphorus amounted to 78 percent or more (samples 10, 12, and 13), gave as good or better results than did superphosphate.

Addition of calcium carbonate at the rate of 2,000 pounds per acre (second series, U. S. Department of Agriculture) did not have a pronounced effect on the weight of millet plants fertilized with either superphosphate, dicalcium phosphate, or calcined phosphate, but caused a marked reduction in the weight of those receiving either colloidal phosphate or untreated Tennessee brown-rock phosphate. Liming increased the total absorption of phosphorus by the plants from the no-phosphorus pots, but decreased the absorption by plants from the phosphate-treated pots.

The highest recovery of the applied phosphorus, as indicated by the percentage absorption of the phosphorus by the plants (table 3), was obtained in the experiments at the Arkansas station, followed in

⁵ The writers are indebted to J. B. Martin, Soil Fertility Investigations, Bureau of Plant Industry, and L. F. Rader, Jr., Fertilizer Investigations, Bureau of Chemistry and Soils, for assistance in making the phosphorus determinations.

order by the experiments at the West Virginia and Alabama stations and the United States Department of Agriculture. Excluding the limed-soil series (U. S. Department of Agriculture), the maximum recovery of the applied phosphorus was 43.9 percent (sample 11, calcined phosphate, Arkansas station) and the minimum recovery was 0.08 percent (sample 7, calcined phosphate, first series, U. S. Department of Agriculture). In general, the percentage recovery of the applied phosphorus decreased with increase in the per acre rate of application of the phosphate.

TABLE 3.—Average oven-dry weight and P_2O_5 content of plants and recovery of applied P_2O_5 ¹

OVEN-DRY WEIGHT

Phosphate treatment		Cabbage, Alabama Experiment Station ²	Millet					Sudan grass	
Sample no.	Material		U. S. Department of Agriculture ³				West Virginia Experiment Station ⁴	Arkansas Experiment Station ⁵	West Virginia Experiment Station ⁶
			First series	Second series		Third series			
				Un-limed	Limed ⁴				
		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
	No phosphorus.....	0.95	3.05	1.85	2.30	5.60	17.8	10.8	5.7
1	Superphosphate.....	16.15	9.25	6.75	6.60	13.45	30.1	32.7	14.5
2	Dicalcium phosphate.....	12.80	10.30	7.90	8.10	16.70	31.1	31.9	16.5
3	Untreated Florida pebble phosphate, 200-mesh.....	14.75	6.20	-----	-----	11.40	30.7	23.7	7.5
4	Untreated Florida pebble phosphate, 100-mesh.....	-----	-----	-----	-----	10.40	-----	-----	7.1
5	Colloidal phosphate.....	-----	3.90	3.80	2.55	-----	-----	-----	-----
6	Untreated Tennessee brown-rock phosphate.....	-----	-----	3.35	2.00	7.55	-----	-----	6.9
7	Calcined phosphate, prepared from Florida pebble.....	3.50	3.70	-----	-----	6.60	23.0	14.7	7.4
8	do.....	11.00	6.05	-----	-----	10.60	26.3	20.1	9.3
9	do.....	14.65	10.50	-----	-----	12.15	27.6	24.3	12.8
10	do.....	17.50	11.50	-----	-----	14.75	30.4	28.3	15.6
11	Calcined phosphate, prepared from Tennessee brown rock.....	15.95	12.25	-----	-----	14.25	28.2	34.6	15.0
12	do.....	17.40	12.70	7.40	6.70	15.45	31.0	30.3	15.8
13	do.....	-----	-----	-----	-----	16.25	-----	-----	-----

AVERAGE P_2O_5 CONTENT¹

		Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams
1	No phosphorus.....	1.4	13.1	7.2	10.1	31.9	49.8	71.6	22.5
2	Superphosphate.....	42.4	48.9	32.4	25.7	70.6	91.8	109.4	58.8
3	Dicalcium phosphate.....	34.8	49.5	34.4	31.5	91.8	100.3	149.9	69.6
4	Untreated Florida pebble phosphate, 200-mesh.....	51.6	25.4	-----	-----	48.1	97.2	136.6	31.4
5	Untreated Florida pebble phosphate, 100-mesh.....	-----	-----	-----	-----	45.2	-----	-----	30.8
6	Colloidal phosphate.....	-----	18.6	14.8	11.2	-----	-----	-----	-----
7	Untreated Tennessee brown-rock phosphate.....	-----	-----	13.7	8.8	41.5	-----	-----	24.4
8	Calcined phosphate, prepared from Florida pebble.....	7.2	13.6	-----	-----	24.1	69.1	92.0	24.6
9	do.....	29.4	23.5	-----	-----	43.0	74.7	121.5	32.6
10	do.....	49.6	35.7	-----	-----	49.8	75.7	143.0	54.9
11	do.....	47.4	49.5	-----	-----	64.2	92.2	187.0	62.7
12	Calcined phosphate, prepared from Tennessee brown rock.....	61.6	45.3	-----	-----	55.6	84.1	218.1	62.6
13	do.....	63.5	67.4	37.3	30.4	67.2	106.6	176.9	67.8
	do.....	-----	-----	-----	-----	73.9	-----	-----	-----

¹ Per pot.

² Total P_2O_5 applied at rate of 0.64 g per pot (180 pounds per acre).

³ Total P_2O_5 applied at rate of 0.80 g per pot (240 pounds per acre).

⁴ C. p. $CaCO_3$ applied at rate of 2,000 pounds per acre.

⁵ Total P_2O_5 applied at rate of 0.35 g per pot (100 pounds per acre).

⁶ Total P_2O_5 applied at rate of 0.334 g per pot (66.8 pounds per acre).

TABLE 3.—Average oven-dry weight and P_2O_5 content of plants and recovery of applied P_2O_5 —ContinuedAVERAGE RECOVERY OF APPLIED P_2O_5

Phosphate treatment		Cabbage, Alabama Experiment Station	Millet					Sudan grass	
Sample no.	Material.		U. S. Department of Agriculture				West Virginia Experiment Station	Arkansas Experiment Station	West Virginia Experiment Station
			First series	Second series		Third series			
				Un-limed	Lime				
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	Superphosphate.....	6.4	6.0	4.2	2.6	6.4	12.0	20.3	10.4
2	Dicalcium phosphate.....	5.2	6.1	4.5	3.6	10.0	14.4	23.4	13.5
3	Untreated Florida pebble phosphate, 200-mesh.....	7.8	2.1	-----	-----	2.7	13.5	19.5	2.5
4	Untreated Florida pebble phosphate, 100-mesh.....	-----	-----	-----	-----	2.2	-----	-----	2.4
5	Colloidal phosphate.....	-----	.9	1.3	.2	-----	-----	-----	-----
6	Untreated Tennessee brown-rock phosphate.....	-----	-----	1.1	- 2	1.6	-----	-----	.5
7	Calcined phosphate, prepared from Florida pebble.....	.9	.08	-----	-----	.4	5.5	6.1	.6
8	do.....	4.4	1.7	-----	-----	1.8	7.1	14.9	3.2
9	do.....	7.5	3.8	-----	-----	3.0	7.4	21.4	9.3
10	do.....	7.2	6.1	-----	-----	5.4	12.1	34.6	11.5
11	Calcined phosphate, prepared from Tennessee brown rock.....	9.4	5.4	-----	-----	3.9	9.8	43.9	11.5
12	do.....	9.7	9.1	5.0	3.4	5.9	16.2	31.5	12.9
13	do.....	-----	-----	-----	-----	7.0	-----	-----	-----

RELATIVE EFFICIENCIES OF THE PHOSPHATES

The relative efficiencies of the phosphates, as indicated by the increase in the dry weight and phosphorus content of the plants, are summarized in table 4. In this table the efficiencies of the phosphates are based upon 100 as the increase in dry weight or phosphorus content due to the application of superphosphate. Although the individual series of experiments showed some discrepancies between the relative efficiencies of the phosphates as determined by the increase in the dry weight and the phosphorus content, respectively, the average efficiencies (table 4), as determined in this way, were, for the most part, in very good agreement. In general, the average efficiency as determined by the increase in dry weight was somewhat higher than that determined by the increase in phosphorus content.

The efficiency of the untreated 200-mesh Florida pebble phosphate rock was superior or approximately equal to that of the superphosphate in the experiments with cabbage at the Alabama station and with millet at the West Virginia station. In the other experiments the efficiency of this material was decidedly inferior to that of superphosphate. The results of the two experiments in which direct comparisons were made indicate a slightly higher efficiency for the 200-mesh Florida pebble phosphate rock than for the 100-mesh material. The colloidal phosphate and the untreated Tennessee brown-rock phosphate were markedly less efficient than the untreated Florida pebble. The relative efficiency of dicalcium phosphate, as indicated by these experiments, was approximately the same as that reported by Ross, Jacob, and Beeson (25).

In the case of the calcined phosphates, removal of only 50 percent of the fluorine (sample 7) caused a reduction in the citrate solubility and a marked decrease in the relative nutrient efficiency of the phosphorus as compared with that of the phosphorus in the original phosphate rock. With further removal of fluorine there was a progressive increase in the citrate solubility and, with few exceptions, in the relative nutrient efficiency of the phosphorus. Calcined phosphates (samples 10 to 13) from which 90 percent or more of the total fluorine had been volatilized (corresponding to a citrate solubility of 65.4 percent or higher) showed higher average relative nutrient efficiencies than did superphosphate.

TABLE 4.—Comparative efficiencies and availabilities of phosphates

Sample No	Phosphate	Average efficiency of P_2O_5 as indicated by increase in —		Availability of P_2O_5 determined by—	
		Dry weight of plants ¹	P_2O_5 content of plants ¹	Neutral ammonium citrate method ²	2-percent citric acid method ²
				Percent	Percent
1	Superphosphate	100.0	100.0	99.6	90.4
2	Dicalcium phosphate	³ 112.4	³ 119.9	100.0	100.0
3	Untreated Florida pebble phosphate, 200-mesh	⁴ 66.6	⁴ 67.1	13.2	21.9
4	Untreated Florida pebble phosphate, 100-mesh	⁴ 38.5	⁴ 28.6	11.6	21.0
5	Colloidal phosphate	⁵ 26.7	⁵ 22.8	10.4	31.7
6	Untreated Tennessee brown-rock phosphate	⁶ 23.0	⁶ 18.6	7.6	19.8
7	Calcined phosphate, prepared from Florida pebble	⁴ 19.7	⁴ 15.6	6.8	16.8
8	do	⁴ 54.9	⁴ 44.5	20.6	27.7
9	do	⁴ 85.9	⁴ 75.2	49.1	54.4
10	do	⁴ 109.4	⁴ 104.5	78.1	82.6
11	Calcined phosphate, prepared from Tennessee brown rock	⁴ 109.4	⁴ 106.6	65.4	69.3
12	do	³ 116.2	³ 125.9	80.1	92.7
13	do	⁷ 135.7	⁷ 108.5	92.7	94.3

¹ Based on the increase from superphosphate as 100; excluding results obtained with limed soils

² Based on the total P_2O_5 content of the material.

³ Average of 7 series of experiments.

⁴ Average of 6 series of experiments.

⁵ Average of 2 series of experiments.

⁶ Average of 3 series of experiments.

⁷ 1 series.

COMPARATIVE EFFICIENCIES AND AVAILABILITIES OF PHOSPHATES

The data given in table 4 show a fairly direct relation between the availability of the phosphorus, as determined by either the neutral ammonium citrate or the 2-percent citric acid method, and the relative nutrient efficiency of the phosphorus, as indicated by the increase in either the dry weight or the phosphorus content of the plants. The relation is particularly noticeable in the series of calcined phosphates (samples 7 to 10) prepared from Florida pebble phosphate. The average relative efficiencies of the calcined phosphates were significantly higher than their solubilities in ammonium citrate and citric acid would indicate.

With the exception of superphosphate, the average efficiency of the phosphate materials was more closely correlated with the solubility of the phosphorus in 2-percent citric acid than in neutral ammonium citrate solution. Likewise, Jacob and Ross (15) report that the 2-percent citric acid method seems to give a better index of the nutrient

value of steamed bone meal, Tennessee brown-rock phosphate, and synthetic calcium phosphates than does the neutral ammonium citrate method. On the other hand, Bartholomew and Jacob (2) have shown that the citrate method gives, in general, a much better indication of the nutrient value of iron and aluminum phosphates than is obtained by the citric acid method. As compared with the citrate solubility, the lower citric acid solubility of the phosphorus in superphosphate (table 4; also, 14, 15) is probably due to the presence of iron and aluminum phosphates, either or both.

RELATION BETWEEN VOLATILIZATION OF FLUORINE AND NUTRIENT VALUE OF CALCINED PHOSPHATE

As compared with the untreated phosphate rock, volatilization of 50 percent of the total fluorine in the rock caused a decrease of approximately 40 percent in the nutrient value of the phosphorus (table 5)⁶. Also, the nutrient value of the phosphorus was decreased about 10 percent by the removal of 64 percent of the fluorine. On the other hand, volatilization of 97 percent of the total fluorine caused an increase of approximately 55 percent in the nutrient value of the phosphorus, as compared with an increase of about 35 percent when the rock was converted into superphosphate.

TABLE 5.—*Relation between volatilization of fluorine and nutrient value of calcined phosphate*

Sample no.	Phosphate material	Average nutrient value ¹ of phosphate as indicated by total—		Fluorine volatilized in preparation of calcined phosphate	
		Dry weight of plants	P ₂ O ₅ content of plants	Total fluorine	F' ₂ fluorine ²
				Percent	Percent
3	Untreated Florida pebble phosphate, 200-mesh	100.0	100.0		
3 ⁷	Calcined phosphate	62.7	59.2	49.9	0
3 ⁸	do.	93.1	85.3	64.2	1.6
3 ⁹	do.	123.1	116.3	78.9	44.0
4 ¹¹	do.	144.8	143.1	³ 90.0	³ 76.0
3 ¹⁰	do.	143.3	142.0	93.3	82.2
4 ¹²	do.	149.6	163.9	³ 97.0	³ 93.0
1	Florida pebble superphosphate	134.3	137.9		

¹ Based on 100 as the total dry weight or P₂O₅ content of plants receiving untreated Florida pebble phosphate; excluding the second series of the Department of Agriculture.

² Fluorine corresponding to the second atom of fluorine in the fluorapatite equivalent of the total phosphorus.

³ Prepared from Florida pebble phosphate.

⁴ Prepared from Tennessee brown-rock phosphate.

⁵ Approximate figure.

In the Florida pebble phosphate rock used in these experiments, the fluorine in excess of that corresponding to the second (F'₂) atom of fluorine in the fluorapatite equivalent of the total phosphorus, amounted to approximately 63 percent of the total fluorine. In agreement with the relation between fluorine volatilization and citrate solubility established in previous work (17, 22, 23), no increase in the nutrient value of the phosphorus occurred until all of the fluorine in excess of the F'₂ fluorine was volatilized.

⁶ It should be noted that the relative nutrient values shown in table 5 are based on the total dry weight and P₂O₅ content of the plants, in contrast to table 4 in which efficiency of P₂O₅ is based on increases of dry weight and P₂O₅ content.

The available evidence indicates that volatilization of fluorine in quantity less than that present in excess of the F'_B fluorine, results in the formation of hydroxyfluorapatite, a compound which is practically insoluble in citrate solution and which has comparatively little value as a source of phosphorus for plant growth. Volatilization of the F'_B fluorine results in the formation of hydroxyapatite, which also is comparatively insoluble in citrate solution. However, at $1,400^\circ\text{C}$. and in the presence of silica, the hydroxyapatite is converted into citrate-soluble phosphate, which is believed to be principally, if not entirely, the high-temperature form of tricalcium phosphate.

SUMMARY

In order to obtain information on the nutrient value of the phosphorus in calcined phosphate, greenhouse pot experiments with cabbage, millet, and Sudan grass were carried out on Cecil clay, Clarksville silt loam, Norfolk loamy fine sand, and Dekalb silt loam soil types, ranging in pH from 4.8 to 6.23. In the calcined phosphates, which were prepared from Florida land-pebble phosphate and Tennessee brown-rock phosphate, the citrate solubility of the phosphorus ranged from 7 to 93 percent, corresponding to the volatilization of 50 to 97 percent of the total fluorine. Comparative experiments were also made with superphosphate, dicalcium phosphate, and ground phosphate rock.

The nutrient value of calcined phosphate was related, more or less directly, to the citrate solubility of the phosphorus, which depended on the amount of fluorine volatilized from the rock during calcination. Volatilization of the fluorine in quantity less than that (about 63 percent of the total fluorine content of the phosphate rock) corresponding to the fluorine in excess of the second atom of fluorine in the fluorapatite equivalent of the total phosphorus, decreased the citrate solubility of the phosphorus, as compared with that of the phosphorus in the original phosphate rock, and markedly reduced the nutrient value of the phosphorus, as indicated by the plant growth and the absorption of phosphorus. Volatilization of 64 percent or more of the fluorine caused a progressive and pronounced increase in the citrate solubility and nutrient value of the calcined phosphate.

In general, calcined phosphates showing citrate solubilities of approximately 78 percent or higher (corresponding to the volatilization of 93 percent or more of the total fluorine content of the original phosphate rock) were as efficient sources of phosphorus for plant growth as were equivalent quantities of total phosphorus from either superphosphate or dicalcium phosphate.

In general, the effect of the phosphates in increasing the dry weight of cabbage, millet, and Sudan grass was related fairly closely to their effect in increasing the quantity of phosphorus absorbed by the plants.

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THE PHOSPHORUS CONTENT AND REQUIREMENTS OF THE FLOUR BEETLE *TRIBOLIUM CONFUSUM* DUVAL, AND A STUDY OF ITS NEED FOR VITAMIN D¹

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INTRODUCTION

Little is known regarding the mineral requirements of insects or their mineral content. Loeb (4)² demonstrated that *Drosophila* requires phosphorus but not calcium. Uvarov (11) summarized the existing knowledge of the phosphorus content of insects, nine species only being listed. *Tribolium* was not mentioned. Sweetman and Palmer (10) showed the feasibility of using *Tribolium* as a test animal for biological analysis. They found that this insect requires vitamin B (undifferentiated complex) in its food, as had been demonstrated for *Drosophila* by Guyénot (as cited by Uvarov (11)), Northrop (6), and Bacot and Harden (1), and for *Ephestia kuehniella* by Richardson (9). Hobson (2) showed that aseptic blowfly larvae require four types of growth factors found in yeast. These factors are an insoluble fraction and three soluble fractions, one heat-labile, probably B₁, and two heat-stable, probably Y and B₂. No need of *Tribolium* for vitamin A could be demonstrated by Sweetman and Palmer, and additions of vitamin D to a normal ration exerted no beneficial effect. However, no experiments have been reported that show whether insects actually do require vitamin D.

MATERIAL AND METHODS

Tribolium confusum was employed in a study of phosphorus requirement, and inasmuch as the normal utilization of this element by vertebrates is greatly influenced by vitamin D, advantage was taken of the opportunity to examine more critically the question as to whether this insect requires vitamin D. The adequacy of the rations tested was measured in a constant physical environment by the length of the larval stage, either from the day-old egg or from hatching to pupation.

In order to secure a sufficient number of individuals for phosphorus analysis, day-old eggs were placed directly in the experimental rations, which had been adjusted to 10 percent moisture, and incubated at a temperature of 32° C. and a relative humidity of 75 percent. At the time of pupation the pupae were removed daily by sifting the culture through a coarse sieve. About 200 live larvae, pupae, or adults and about 4,000 eggs (figuring 450 per 0.1 cc) represented a single sample for analysis. Dry-matter determinations were made at 100° C. under reduced pressure in vacuo, and total ash, calcium, and phosphorus were determined by the method of Morris, Nelson, and Palmer (5).

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² Reference is made by number (*italic*) to Literature Cited, p. 852.

EXPERIMENTAL RESULTS

Preliminary experiments had indicated that the adults contained more phosphorus than the pupae, and that this increase was taking place in the pupae before metamorphosis was completed.

The results of the analyses of all the stages, given in table 1, show that the percentage of dry matter decreases with advancing stages in the life cycle and that the percentage of ash and phosphorus in the dry matter increases.

Table 2 gives the results of growing *Tribolium* on irradiated and nonirradiated rations of different sorts. When irradiated and nonirradiated farina (with and without added CaCO_3) were used, no differences in the growth of *Tribolium* were observed. When a synthetic irradiated diet was employed the time required for pupation was greater than when a nonirradiated diet was used. Adding cod-liver oil to a nonirradiated ration decreased the time of pupation slightly, but not significantly. The slight benefit of cod-liver oil was more likely due to the alleviation of a dusty condition of this ration than to its vitamin content.

TABLE 1.—Mean analysis of different stages in the life cycle of *Tribolium confusum* when kept at a temperature of 32° C. and a relative humidity of 75 percent, whole-wheat flour, sifted through no. 6 bolting cloth, being used as food

Stage	Individual live weight	Dry matter	Content on a dry-matter basis of—		
			Ash	Calcium	Phosphorus
	Milligrams	Percent	Percent	Percent	Percent
Egg.....	0.092	58.80	1.85	0.024	0.445
Larval.....		42.26	2.07	.040	.456
Pupal.....	2.0	40.01	2.15	.066	.522
Adult.....		39.07	2.36	.054	.598

TABLE 2.—Growth of *Tribolium confusum* on rations containing or deficient in vitamin D with and without a calcium supplement, when kept at a temperature of 32° C. and a relative humidity of 75 percent

[Approximately 1,100 day-old eggs were placed in 300 g of food]

Ration	Phosphorus in ration	Calcium in ration	Pupae	Period of growth, egg to pupation	
				Mean ¹	σ ²
	Percent	Percent	Number	Days	
Irradiated farina ³	0.087	0.030	262	51.5±0.429	10.3±0.303
Nonirradiated farina.....	.087	.030	338	51.3±.348	9.5±.246
Irradiated farina+1.5 percent CaCO_3069	.644	243	56.5±.407	9.4±.288
Nonirradiated farina+1.5 percent CaCO_3069	.644	227	56.1±.145	3.2±.102
Irradiated synthetic diet ⁴196	.527	266	74.1±.339	8.2±.240
Nonirradiated synthetic diet ⁴196	.527	367	60.8±.214	6.1±.151
Nonirradiated synthetic diet ⁴ +0.5 percent cod-liver oil.....	.196	.527	504	55.0±.182	6.1±.129

¹ Mean = $\frac{\sum x}{N}$; \pm Probable error $\bar{x} = 0.6745 \frac{\sigma x}{\sqrt{N}}$

² σ = Standard deviation = $\sqrt{\frac{\sum x^2}{N} - \bar{x}^2}$; \pm Probable error $\sigma_x = 0.6745 \frac{\sigma x}{\sqrt{2N}}$

³ Ration irradiated for 15 minutes at 20 inches with a quartz mercury vapor sun lamp, operating at 110 volts, 5 amperes.

⁴ Synthetic diet contained 12.5 percent arachin, 2.5 percent edestin, 3 percent Crisco, 75.5 percent dextrin, 4 percent Osborne and Mendel (?) salt mixture with only one-half the normal amount of phosphorus present, and 2.5 percent of yeast extract made by digesting pure dry yeast with 20-percent ethyl alcohol for 2 days at 32° C. The arachin was prepared from defatted peanut meal by the method of Johns and Jones (3) and the edestin from defatted hempseed by the sodium benzoate method of Reeves (8).

Inasmuch as the lowest phosphorus content of these rations proved to be essentially the limiting level of this element it is evident that *Tribolium* does not have a vitamin D requirement analogous to that of the vertebrates. The similar growth on similar rations of the high calcium low phosphorus type, on which rats would become so rachitic unless vitamin D is provided, lends further support to this conclusion.

The phosphorus requirement of *Tribolium* was determined from the growth and phosphorus content of the insect when reared on a synthetic ration containing five definite levels of phosphorus. These levels were obtained by using the synthetic ration described in table 2 and five salt mixtures prepared by the Osborne and Mendel (7) technic, one being phosphorus free. From the results given in table 3 it is seen that under the conditions described the time of pupation was proportional to the amount of phosphorus present in the ration. The limiting amount of phosphorus is probably about 0.1 percent. The difference between the time of pupation on 0.1- and 0.2-percent levels of phosphorus was 35 days, which was 248 times the probable error of the difference. However, there was no significant difference in the amount of phosphorus in the pupae. The data indicate that the larvae must eat sufficient food to bring their phosphorus content up to a certain level before pupation will occur. This requirement for phosphorus probably explains in part the slower development and smaller populations of *Tribolium* in patent flour and similar products than in whole-wheat flour. Patent flour ranges in phosphorus content from 0.07 to 0.10 percent.

TABLE 3.—Growth and phosphorus content of *Tribolium confusum* on synthetic rations with different phosphorus levels, when kept at a temperature of 32° C. and a relative humidity of 75 percent

[Approximately 8,100 day-old eggs were placed in 1,500 g of rations 1, 4, and 5, 2,700 in 500 g of ration 2, and 5,400 in 848 g of ration 3]

Ration ¹	Phosphorus content	Pupae	Period of growth, egg to pupation		Phosphorus in dry pupae	
			Mean	σ	Mean	σ
	Percent	Number	Days		Percent	
1	0.017	995	89.96±0.387	18.09±0.274	0.517±0.003	0.009±0.002
2	.107	775	71.49±.138	5.71±.098	.539±.002	.006±.001
3	.205	4,346	35.95±.038	3.72±.027	.530±.002	.015±.002
4	.296	6,881	34.25±.029	3.60±.021	.535±.001	.012±.001
5	.336	7,335	33.43±.026	3.27±.018	.540±.001	.011±.001

¹ Synthetic ration the same as in table 2, the only variable being the amount of phosphorus in the salt mixture.

SUMMARY AND CONCLUSIONS

Analyses of *Tribolium confusum* Duval at different stages in its life cycle show that the eggs, larvae, pupae, and adults contain, respectively, 58.8, 42.26, 40.01, and 39.07 percent of dry matter. The dry matter of eggs, larvae, pupae, and adults contains, respectively, 1.85, 2.07, 2.15, and 2.36 percent of ash, and 0.445, 0.456, 0.522, and 0.598 percent of phosphorus.

Tribolium does not need vitamin D for any physiologic function that can be detected by length of time to pupation or by phosphorus content of the pupae.

The time of pupation of *Tribolium* is somewhat proportional to the amount of phosphorus in the ration, other factors being constant. The limiting amount of phosphorus is probably 0.1 percent. However, the percentage of phosphorus in the pupae is constant regardless of the amount of phosphorus in the ration.

The slower development and smaller populations of *Tribolium* in patent flour and similar products than in whole-wheat flour is probably due in part to the low phosphorus content of these foods.

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VITAMIN A CONTENT OF EGGS PRODUCED BY CHICKENS FED VIOSTEROL AND VARIOUS PERCENTAGES OF COD-LIVER OIL¹

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INTRODUCTION

Very little is known regarding the interrelationships of any of the vitamins either in man or in experimental animals. Much of what little work has been done has given results of a negative nature. Coward (3),² however, has recently reported data which apparently indicate that with rats the growth responses to different doses of one vitamin are influenced by the amount of another vitamin present in the diet. In proof of this she shows that curves of response—average gain in weight plotted against units of vitamin D fed—obtained by giving the same series of doses of vitamin D but with basal diets containing different amounts of vitamin A do not coincide at any point. Similar findings are reported for the B vitamins.

Recent work in this laboratory has shown that the vitamin D content of eggs may be increased many times by adding viosterol to the diet of the chicken (*Gallus domesticus*). It is common knowledge that the vitamin A content of eggs depends upon the amount of this factor in the diet of the chicken. In view of the possibility that vitamin D in the form of viosterol might have some effect on the vitamin A storage in the eggs, it seemed advisable, before definitely recommending that poultry raisers use viosterol to increase the vitamin D content of eggs, to determine what effect, if any, this added vitamin D might have on the vitamin A content of the egg. Cod-liver oil was used in the diet as the source of vitamin A. Incidental to this study of vitamin interrelationships data were obtained on the influence of the various levels of cod-liver oil in the diet of the chicken on vitamin A content of egg yolk.

REVIEW OF LITERATURE

Data on the vitamin A content of eggs have been reported by various workers. Sherwood and Fraps (9) have recently shown that eggs from pullets fed a basal diet containing 20 percent yellow corn as the only source of vitamin A had about 20 Sherman-Munsell units of vitamin A per gram of yolk at the beginning of the experiment and from 5 to 8 toward the end of the 6½-month feeding period.

Bisbey and coworkers (2) reported the influence of yellow corn, and yellow corn plus alfalfa or cod-liver oil in the diet of the chicken on the vitamin A content of the egg yolk. The percentage of these components in the diets and the vitamin A content of the egg yolk

¹ Received for publication Mar. 7, 1935; issued July, 1935

² Reference is made by number (italic) to Literature Cited, p. 856.

were as follows: On 65 percent of yellow corn plus 10 percent of alfalfa, 28 units (Sherman-Munsell) per gram of yolk; on 65 percent of yellow corn, 9 units; on 35 percent of yellow corn, 7 units; and on 25 percent of yellow corn plus 0.5 percent of cod-liver oil, 28 units.

Munsell (7) reported the vitamin A content of some eggs purchased on the New York market as 50 units per gram of yolk.

Bethke, Kennard, and Sassaman (1) gave the vitamin A value of yolks of eggs from confined birds as varying from 5.2 to 11.4 units, while that of eggs from birds on range or receiving cod-liver oil had 25 units or more per gram.

MATERIALS AND METHODS

Three pairs of pens of crossbred pullets kept at the United States Animal Husbandry Farm at Beltsville, Md., were used in the present experiment. These birds were the offspring resulting from mating Barred Plymouth Rock females with Rhode Island Red males. Each pen contained 18 pullets and 1 cockerel. The birds were confined indoors and received no direct sunlight. A description of the basal diet and the vitamin supplements are given below.

Basal diet fed to paired pens of pullets

	Percent		Percent
Basal feed mixture.....	73. 15	Ground limestone.....	2. 87
Desiccated meat meal.....	8. 00	Special steamed bone meal.....	2. 98
North Atlantic whitefish meal..	7. 00	Anhydrous Na_2SO_4 50
Dried buttermilk.....	5. 00	NaCl 50

Vitamin supplements given to paired pens of pullets

- Pen 21—1 percent of cod-liver oil.
- Pen 22—1 percent of cod-liver oil and 0.5 percent of viosterol 160 D.
- Pen 23—2 percent of cod-liver oil.
- Pen 24—2 percent of cod-liver oil and 0.5 percent of viosterol 160 D.
- Pen 25—8 percent of cod-liver oil.
- Pen 26—8 percent of cod-liver oil and 0.5 percent of viosterol 160 D.

The basal feed mixture which made up the bulk of the basal diet contained yellow corn meal, 52.63 percent; wheat bran, 25.79 percent; rolled oats, 15.79 percent; alfalfa-leaf meal, 5.79 percent. The cod-liver oil was a high-grade commercial product sold for animal-feeding purposes. The pullets were put on the above-described diets at the end of August 1933. Egg samples for analysis were taken from January 1 through June. Each sample was made up from the eggs laid during a 7-day period and included one egg from each pullet that laid.

In addition to eggs from pullets in the 6 pens listed above, a composite sample of eggs from 8 pens of pullets fed diets which contained no added cod-liver oil or viosterol was tested for vitamin A. The diets fed to these 8 pens of pullets contained the same percentage of basal feed mixture as the basal diet described above but the protein supplements in each case were entirely of plant origin. The birds on these diets had access to a limited range. The eggs will be referred to as from pullets on a. p. s. (all plant source) diet.

Vitamin A tests were made according to the regular Sherman-Munsell method (8) except that the test period was of 5 weeks' duration instead of the usual 8 weeks. The basal diet fed to the rats

was a slight modification of that used by Sherman and Munsell. It contained the following ingredients: Vitamin-A-free casein, 18 percent; irradiated yeast, 5 percent; nonirradiated yeast, 5 percent; Osborne and Mendel salts, 4 percent; sodium chloride, 1 percent; hydrogenated vegetable fat, 10 percent; and starch, 57 percent. The rats were 21 days old when put on the basal diet. They showed cessation of growth and quite uniform ophthalmic symptoms about the thirty-first day on this diet.

Samples of egg-yolk mixture for testing were made up each week. The yolks were separated as cleanly as possible from the whites and any small amount of white still adhering was removed with filter paper. The yolks were then mixed thoroughly with an electric beater and a weighed portion of the composite sample mixed with four times its weight of vitamin-A-free casein. These egg-casein mixtures were weighed out on an analytical balance three times weekly, and fed as supplements to the basal diet. Between weighings they were stored in air-tight containers at 20° F.

EFFECT OF VIOSTEROL

The main purpose of the experiment was to determine whether there was any difference in vitamin A storage in the eggs due to added vitamin D in the diet. Therefore equal weights of yolk of eggs from pullets given cod-liver oil and from those given the same quantity of cod-liver oil with viosterol were allotted to litter-mate test animals of the same sex and weight. For example, when a rat was allotted 0.05 g per day of yolk of eggs from pen 21 (table 1) a carefully matched animal of the same litter, sex, and weight was allotted the same amount of yolk of eggs from pen 22.

TABLE 1.—Comparison of the gain in weight and the food intake of paired groups of rats having as the only source of vitamin A the yolk of eggs from pullets receiving cod-liver oil or cod-liver oil and viosterol

Pairs of rats fed egg yolk (number)	Quantity of egg yolk fed per rat per day	Eggs obtained from pen no.	Supplement in diet of pullets		Total gain in weight of rats during 5-week test period			Total food intake of rats							
			Cod-liver oil	Viosterol 160 D	Average per rat	Standard deviation of differences between pairs of rats	Probability that differences may have occurred by chance	Average per rat	Standard deviation of differences between pairs of rats	Probability that differences may have occurred by chance					
	Grams		Pct.	Percent	Grams			Grams							
10.....	0.0125	21	1	0	(1)	22.96	0.47	{ 241.4 235.4 219.7 240.2 254.0 246.1 297.1 285.8 263.3 271.4 276.4 279.6 304.7 309.3 }	48.96	0.65					
		22	1	.5											
10.....		23	2	0	(1)										
10.....		24	2	.5											
14.....	.025	25	8	0	19.9	19.93	.15		34.95	.10					
		26	8	.5	15.3										
10.....		21	1	0	6.2										
10.....		22	1	.5	16.3										
11.....	.05	23	2	0	21.2	13.10	.53	29.47	.41	.19					
		24	2	.5	18.7										
15.....		25	8	0	48.4										
15.....		26	8	.5	38.9										
17.....	.05	21	1	0	28.2	17.18	.89	46.30	.80	.63					
		22	1	.5	27.5										
14.....		23	2	0	34.4										
14.....		24	2	.5	35.0										
15.....		25	8	0	52.9	15.51	.63	{ 304.7 309.3 }	35.98						
15.....		26	8	.5	54.9										

¹ 13 of the 20 rats died before the end of the 5-week test period.

The egg samples were also allotted to the members of a litter of test animals in such a way that all (up to six) received the same weight of egg mixture but each from a different pen. Therefore comparisons of the growth of animals on the same weight of egg from the various diets are comparisons, for the most part, between litter mates, while those between rats fed different amounts of the same kind of egg are not litter-mate comparisons. Furthermore, the eggs were apportioned in such a way that any seasonal influence would be equalized in the tests of all of the different kinds of eggs and the different levels of feeding.

Feeding trials of this kind with several pairs of rats were made at three levels, 0.0125, 0.025, and 0.05 g. Most of the animals fed 0.0125 g daily of yolk of eggs from pens 21, 22, 23, and 24 died before the end of the 5-week test period. Therefore no data were available for analysis at this level of the eggs from these two pairs of pens.

The effect of added vitamin D in the diet of the chicken on the vitamin A content of the egg yolk was evaluated by comparing the differences between the gains in weight made by the rats of each pair by Student's method as described by Fisher (5). The formula used is

$$t = \frac{\text{mean difference} \times \sqrt{N}}{S},$$

where S is the observed standard deviation of the differences and N is the number of pairs. A t value corresponding to a probability of 0.01 is to be considered as indicating a significant average difference. The mean gains, food intake, number of pairs used in each comparison, the standard deviation, and the probability of getting a difference as great or greater than the observed difference are tabulated in table 1.

Since only one of the differences has a probability that approaches 0.01 and the rest are greater than 0.05, it may be concluded that the presence of added vitamin D in the diet of the chicken had no significant influence on vitamin A storage in the eggs.

EFFECT OF DIFFERENT LEVELS OF COD-LIVER OIL

Comparisons were also made of the vitamin A content of the yolks of eggs from pullets on the various levels of cod-liver oil and those on the all-plant-source diets. Data from rats fed the same weight of egg yolk from the two groups of pullets on each level of cod-liver oil were combined irrespective of whether the diet contained added viosterol. For example, the data from rats fed 0.025 g of yolk of eggs from pen 21 were combined with the data from rats receiving a like amount of yolk of eggs from pen 22. This seemed a logical procedure since no effect on storage of vitamin A in eggs due to viosterol in the diet of the pullet could be demonstrated. A few additional values were included that were not used in the paired comparisons of the first analysis either because the animal from which the data were obtained was an odd one of a litter or because its litter mate had died. Each group, however, contained equal numbers of males and females.

These comparisons were made by covariance analysis. The gains in weight were corrected for differences in feed intake by means of the regression of gain in weight on feed eaten. Snedecor (10) and Tippet

(11) describe the mechanics of this method. Applications of covariance analysis to biological data are given in detail by Titus and Harshaw (12) and by Crampton (4).

As already explained, data were available from only seven of the groups of rats fed eggs from pullets given cod-liver oil. These 7 groups as well as the 2 groups fed eggs from pullets given no added vitamin concentrate were regarded as separate treatments. An analysis of covariance was considered justifiable since the slopes of the regression lines expressing the relation between feed and gain were approximately the same for all of the nine groups. The variance was divided only into that attributable to treatment and to error. Using the pooled regression coefficient of 0.33204, the sums of squares were corrected and a z value [$z = \frac{1}{2} (\log_e \text{variance due to treatment} - \log_e \text{variance due to error})$] of 1.1188 was obtained. The necessary z for $P=0.01$ when $n_1=8$ and $n_2=254$, is 0.4742. Since the calculated value of z is greater than this, it is probable that there are significant differences among the corrected gains which are not attributable to chance. The mean gain in weight of the rats of each group was then corrected by subtracting the product of the regression coefficient and the difference between the mean food intake of the group and the mean food intake of all of the groups (266.8 g). The corrected values are given in table 2.

TABLE 2.—Observed and corrected average gains in weight of the groups of rats used in the vitamin A tests on yolk of eggs from pullets fed different percentages of cod-liver oil

Rats in group (number)	Quantity of egg yolk fed per rat per day as source of vitamin A	Eggs ob- tained from pen no.---	Cod-liver oil in diet of pullets	Average gain in weight per rat (5 weeks)	Average food intake per rat (5 weeks)	Average corrected gain in weight per rat (5 weeks)
	Grams		Percent	Grams	Grams	Grams
28.....	0.0125	25-26	8	17.6	238.6	26.9
30.....	.025	21-22	1	11.3	231.4	23.1
30.....		23-24	2	19.5	244.7	26.8
38.....		25-26	8	44.1	292.1	35.7
36.....	.050	21-22	1	29.2	268.7	28.5
38.....		23-24	2	34.4	274.7	31.8
30.....		25-26	8	54.1	306.4	40.9
18.....	.080	(1)	0	19.0	246.6	25.7
16.....	.10	(1)	0	43.7	289.0	36.3

¹ All-plant-source diet.

In table 3, treatment differences between any two groups are summarized. The significance of the differences was evaluated by means of the t test. A t value corresponding to a probability of 0.05 is considered significant and 0.01 highly significant. The number of degrees of freedom (n) is 254, since that number was used in estimating the error variance.

The results of the analysis may be summarized briefly. The eggs from birds fed 1 and 2 percent of cod-liver oil were significantly different in vitamin A content from those fed 8 percent. Since an equivalent weight of the yolks of eggs from the latter pair of pens produced gains in weight uniformly greater than an equal amount of yolk of eggs from either of the other two pairs of pens, it is concluded that the eggs from the birds fed 8 percent of cod-liver oil were richer

in vitamin A than the others. Additional evidence of the greater vitamin A content of the eggs from birds on the high cod-liver oil diets was the survival of most of the rats fed the 0.0125-g portion of the yolks of eggs from these pens and the fact that the majority of the rats fed the same amount of yolk from the other two pairs of pens died. No significant difference in vitamin A content was found between the eggs from the two groups fed 1 and 2 percent of cod-liver oil. However, since differences in gains in weight of the test animals fed equal quantities of egg yolk were always positive in favor of the pullets receiving 2 percent of cod-liver oil, it is probable that if larger numbers of animals had been used in making the vitamin tests a significant difference between these two levels of cod-liver oil might have been demonstrated. The eggs from the birds fed no vitamin A supplement were significantly different in vitamin A content from those of the birds fed cod-liver oil. Since the gains in weight of the rats fed eggs from the birds receiving no cod-liver oil were uniformly inferior it is concluded that these eggs were poorer in vitamin A than any of the others.

TABLE 3.—Significance of differences ¹ in average gains in weight of groups of rats having as the sole source of vitamin A yolk of eggs from pullets fed different percentages of cod-liver oil

Quantity egg yolk per rat per day (grams)	Eggs obtained from pen no.---	Results when given quantity of egg yolk from indicated pen number was fed per rat per day									
		0 0125 g	0 025 g				0.050 g			0 05 g	0 10 g
		25-26	21-22	23-24	25-26	21-22	23-24	25-26	A. p s ¹	A p s ²	
0.0125-----	25-26		+ N	+ N	- H	- N	- S	- H	+ N	- H	
	21-22	- N		- N	- H	- S	- H	- H	- N	- H	
0.025-----	23-24	- N	+ N		- H	- N	- S	- H	+ N	- H	
	25-26	+ H	+ H	+ H		+ H	+ N	- S	+ H	- N	
	21-22	+ N	+ S		- H		- N	- H	+ N	- S	
0.050-----	23-24	+ S	+ H	+ S	- N	+ N		- H	+ S		
	25-26	+ H	+ H	+ H	+ S	+ H	+ H		+ H	+ N	
0.050-----	(?)	- N	+ N	- N	- H	- N	- S	- H		- H	
0.10-----	(?)	+ H	+ H	+ H	+ N	+ S	+ N	- N	+ H		

¹ A difference that can be expected to occur by chance only 1 in 100 times is called highly significant and is indicated by the letter H; one that may occur only 1 in 20 times is called significant, S; and one that would not occur by chance more often than 1 in 20 times is called not significant, N. A positive sign at the side of a letter indicates that the difference is in favor of the pen listed at the left; a negative sign indicates that the difference is in favor of the pen listed in the box head at the top of column.

² All-plant-source diet.

TABLE 4.—Vitamin A content of yolk of eggs from pullets receiving different percentages of cod-liver oil or cod-liver oil and viosterol

Eggs obtained from pen no.—	Supplement in diet of pullets		Vitamin A units per gram of egg yolk (Sherman-Munsell units)	Eggs obtained from pen no.—	Supplement in diet of pullets		Vitamin A units per gram of egg yolk (Sherman-Munsell units)
	Cod-liver oil	Viosterol 160 D			Cod-liver oil	Viosterol 160 D	
	Percent	Percent			Percent	Percent	
21.....	1	0	(1)	25.....	8	0	80
22.....	1	.5	(1)	26.....	8	.5	80
23.....	2	0	40	A. p. s. ²	0	0	* 20
24.....	2	.5	40				

¹ Slightly less than 40.

² All-plant-source diet.

In table 4 are listed the approximate number of Sherman-Munsell units of vitamin A in the yolks of the eggs from the various sources.

CONCLUSIONS

Vitamin D in the form of 0.5 percent of viosterol 160 D in the diet of the chicken (*Gallus domesticus*) receiving graded quantities of cod-liver oil had no apparent effect on the transfer of vitamin A to the egg.

Eggs from pullets fed 8 percent of cod-liver oil were several times richer in vitamin A than eggs from pullets fed 1 and 2 percent of cod-liver oil. These findings should not necessarily be interpreted to mean that in ordinary poultry practice it is desirable to increase the cod-liver oil content of the diet to an 8-percent level in order to increase the vitamin A content of the eggs. It has been definitely shown (6) that the optimum level of cod-liver oil feeding in the diet of the chicken is between 1 and 2 percent. When this level is exceeded impairment in egg production and hatchability is likely to occur.

Although no significant difference could be demonstrated between eggs from birds fed 1 and 2 percent of cod-liver oil, it is probable that had more test animals been used a significant difference in favor of the 2-percent group might have been shown.

The vitamin A potency of eggs from pullets on diets containing no vitamin A and vitamin D supplement was significantly less than the potency of eggs from any of the groups given cod-liver oil. The eggs from these pullets were judged to be about one-fourth as potent as eggs from birds fed 8 percent of cod-liver oil and about one-half as potent as those from birds fed 1 or 2 percent of cod-liver oil. The eggs from birds receiving no vitamin A and B supplement had about 20 units (Sherman-Munsell) of vitamin A per gram of egg yolk, while the eggs from birds fed 8 percent of cod-liver oil had about 80 units, and those from pullets fed 1 or 2 percent of cod-liver oil about 40 units.

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EFFECT OF TEMPERATURE AND LIGHT ON DEVELOPMENT OF THE UREDIAL STAGE OF PUCCINIA GRAMINIS¹

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INTRODUCTION

One of the important problems in the study of the epidemiology of stem rust, *Puccinia graminis* Pers., concerns the extent to which the uredial stage can persist through the winter under different conditions. The ability of this stage to survive obviously depends upon the ability of the urediospores or of the uredial mycelium in grains and grasses to withstand fluctuating temperatures and other conditions during the winter. It seems clear from the investigations of Stakman et al., as cited by Lambert (12),³ that *P. graminis* does not commonly overwinter in the uredial stage north of Texas. As temperature, moisture, and light are likely to be among the most important physical factors involved, experiments were made to determine their effect.

HISTORICAL REVIEW

In the northern part of the United States the fate of the uredial stage of *Puccinia graminis* in the winter has been studied many years. In March 1905 Bolley and Pritchard (4) collected viable urediospores from leaves of quackgrass and wild barley frozen in the ice at Fargo, N. Dak. In 1906-07 Freeman and Johnson (6) were able to find viable urediospores on *Hordeum jubatum* L. and *Agropyron repens* (L.) Beauv. as late as April 15. In 1911 Hungerford (10) showed that urediospores of timothy stem rust, *P. graminis* *phlei-pratensis* (Eriks. and Henn.) Stak. and Piem., persisted through the winter in Wisconsin, while Stakman and Piemeisel (24) observed that they "survived the very severe winter of 1916-17 at St. Paul, Minn., very easily." It is evident, however, that *P. graminis* *phlei-pratensis* differs from other varieties of *P. graminis* in that its uredial stage can survive the winter in the northern United States more easily

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² Now in the Division of Plant Disease Eradication and Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Acknowledgment is made to Dr. E. C. Stakman, University of Minnesota, for suggesting the problem and for helpful criticism during its prosecution; to Dr. R. B. Harvey, University of Minnesota, for suggestions relative to some of the physiological phases; and to Dr. M. N. Levine, Division of Cereal Crops and Diseases, for criticism and suggestions. The writer is also indebted to Dr. Stakman and his coworkers for the cultures of *Puccinia graminis* and other material and to H. G. Ukkelberg, University Farm, St. Paul, Minn., for assistance in some of the routine work.

³ Reference is made by number (italic) to Literature Cited, p. 879.

than can that of other varieties. Lambert (12, p. 27) has summarized some of the extensive studies of Stakman et al. made—

throughout the Mississippi Valley to determine the northern limits of overwintering of *P. graminis tritici* in the uredinal stage. * * * In general, they indicate that, north of Oklahoma, primary infection of cereals as a result of local overwintering in the uredinal stage, occurs so rarely that it is probably of no practical importance.

Hungerford (11) could not confirm the theory of Pritchard (19, 20) that the mycelium of *P. graminis* overwintered in seed wheat as mycelium and that when the seed germinated the mycelium grew by penetrating "various parts of the seedling, invading both the intercellular spaces and the cells."

It seems clear, therefore, that the uredial stage of most of the varieties of *Puccinia graminis* investigated does not commonly survive the winter in the northern United States. The question then arises whether urediospores and the uredial mycelium in perennial grasses and winter grains are killed by the cold or by alternate freezing and thawing. In an attempt to answer this question experiments were made to determine the resistance of urediospores and mycelium to low temperature as well as to fluctuating temperature. It is possible that the ability of urediospores to withstand extremely low temperature may depend somewhat upon the temperature and light conditions that prevailed prior to freezing. Experiments also were made, therefore, to determine the effect of temperature and light on the development of the uredial stage.

MATERIALS AND METHODS

Experiments on the effect of temperature and light were made in four large chambers kept at constant temperatures regulated by a large, automatically controlled refrigerating machine. The chambers were approximately 10 feet square and were lighted artificially by 1,000-watt incandescent lamps. To maintain uniform temperature and humidity, electric fans were kept running continuously. In addition to the four temperature chambers, an ice box cooled by a special refrigerating machine was used for extremely low temperatures. The greenhouse in which the plants were incubated in moist chambers was kept at about 20° C. by means of automatic heat controls.

Temperatures were recorded every 10 minutes by a Leeds-Northrop electrical recording machine and deviations from the desired temperature were corrected immediately, although the variation usually was not more than +1° C.

Several physiologic forms of *Puccinia graminis tritici* Eriks. and Henn., *P. graminis avenae* Eriks. and Henn., and *P. graminis secalis* Eriks. and Henn., and one collection each of *P. graminis phlei-pratensis* and *P. triticea* Eriks. were used. The histories of these forms are shown in table 1. The forms studied, except those of *P. graminis phlei-pratensis* and *P. triticea*, were cultured on some or all of the differentials of wheat, oats, and rye used in identifying the forms (1, 5, 23). The spore measurements were made under the 4-mm objective by means of a Zeiss ocular screw micrometer attached to a Spencer microscope.

Urediospores were hardened by placing plants heavily infected with the uredial stage in the 0° C. room for at least 10 days. This process was the same as that used by Harvey (9) for hardening higher plants before subjecting them to subzero temperatures.

TABLE 1.—*History of varieties and physiologic forms of Puccinia graminis and P. triticina used in studies of the effect of temperature and light on the uredial stage*

Species, variety, and form	Original host	Date collected	Locality in which collected	Collector
<i>P. graminis tritici</i> :				
Form 15	Wheat	May 12, 1923	Pusa, India	R. R. Sen.
Form 35	do.	Sept. 2, 1926	St. Paul, Minn.	E. Miller.
Form 36	do.	Aug. 22, 1924	Morris, Minn.	M. N. Levine.
<i>P. graminis avenae</i> :				
Form 5	Oats	Aug. 13, 1929	Morrisville, N. Y.	Do.
Form 2	do.	Aug. 17, 1929	Mankato, Minn.	R. H. Bamberg, Lee Person.
<i>P. graminis secalis</i> :				
Form 7 (brown)	<i>Agropyron repens</i>	Aug. 9, 1927	St. Peter, Minn.	C. L. Lefebvre et al.
Form 7 (orange)	Rye.	Mar. 3, 1929	Greenhouse, University Farm, St. Paul, Minn.	L. W. Melander.
<i>P. graminis phlei-praetensis</i>	Timothy	Sept. 25, 1929	Scott County, Minn.	Do
<i>P. triticina</i>	Wheat	1930	St. Paul, Minn. ¹	

¹ Originated apparently as a mutation from the brown culture of *Puccinia graminis secalis* form 7; according to Cotter and Levine (5), there was no difference in the pathogenicity of the two strains.

² Contamination in greenhouse.

With few exceptions, the method of inoculation and incubation was uniform and similar to the one described by Cotter and Levine (5). Seedling plants first were sprayed with water and then inoculated by brushing them with rusted plants. The inoculated plants were placed in incubation chambers, again sprayed with water, kept at a temperature of 20° C. for 48 hours, and then placed under the conditions provided for a given experiment. All plants were watched closely for the first appearance of flecks and uredia. When the uredia had reached their maximum development, notes were taken on the number of plants inoculated and infected and on the type of infection produced.

Several experiments failed in the low light intensities because root rot killed the plants before rust pustules appeared. Thereafter all seed was treated with Ceresan⁴ to delay damage from root rot until after uredia had developed and final notes had been taken.

RESULTS

EFFECT OF TEMPERATURE

In general, a temperature of 20° C. seems most favorable for the development of the uredial stage of *Puccinia graminis*, while subzero centigrade temperatures may kill urediospores, especially when moist, and temperatures just above zero may retard development by lengthening the incubation period and by preventing growth and sporulation. The effect of temperature may vary with varieties and physiologic forms. An account of the results of controlled experiments on these phases of the problem follows.

EFFECT OF CONSTANT SUBZERO CENTIGRADE TEMPERATURES ON VIABILITY OF UREDIOSPORES

The effect of outdoor conditions in winter on the viability of urediospores has been studied by various investigators (3, 4, 6, 17, 18, 20),

⁴ An organic mercury compound used in the treatment of seed for the prevention of numerous seed-borne diseases.

but very little is known concerning the effect of temperatures below 0° C. exclusive of other factors. Mehta (16) found that—

* * * after exposure to -6.5° to -10° C. for 24 hours, the urediniospores of this rust (*Puccinia graminis*) do not show even 10 percent germination and after four to seven days there is a total loss (only one or two spores germinating) of viability.

In the present work urediospores were exposed to temperatures of -29° to -40° C. constantly for various periods. The question arose whether hardening had any effect on the viability of urediospores. This is important because, in the Northern States, urediospores are exposed in the fall to temperatures just above 0° before the onset of cold winter weather. That fungi can be hardened has been demonstrated. Bartetzko (2), in 1909, showed that the minimum temperatures withstood by *Aspergillus niger* Van Tieg. could be lowered by first hardening, or exposing the fungus to a temperature just above freezing.

Numerous investigators (4, 5, 6) have observed that urediospores of *Puccinia triticina* live over the winter in the northern United States. In view of this fact, urediospores of this rust were frozen along with those of *P. graminis tritici*, *P. graminis avenae*, and *P. graminis phlei-pratensis*.

Spores of approximately the same age were dusted on dry glass slides in individual small pasteboard boxes. These were placed in an ice box maintained at temperatures below -29° C. Germination tests were made at the end of 1, 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, and 45 days. Hardened spores were obtained from plants kept at 0° to 1° for at least 10 days after the uredia were mature, and nonhardened spores from plants inoculated at the same time but kept continuously in the greenhouse. After exposure to low temperatures, the spores to be tested were placed in a drop of distilled water on a glass slide and kept at 20° for 24 hours, after which counts were made.

That some urediospores of the *tritici* and *phlei-pratensis* varieties of *Puccinia graminis* can survive temperatures ranging from -29° to -40° C. for at least 45 days is shown in table 2. It appears that because of the sudden change in temperature more spores are killed during the first 24 hours than during any subsequent period. The viability of hardened spores of *P. graminis tritici* (table 2) was least affected by low temperature, and nonhardened spores of *P. graminis avenae* form 2 succumbed most readily, being unable to withstand the low temperature for more than 20 days in 2 out of 3 trials.

Hardened spores of *Puccinia graminis* withstood temperatures of -29° to -40° C. better than nonhardened spores (table 2 and fig. 1). While very few spores of *P. graminis avenae* survived, hardening seemed to enable some of them to survive prolonged exposure to -29° to -40°. There seemed to be little or no difference in ability of hardened and nonhardened urediospores of *P. triticina* to withstand low temperature.

Clearly, some urediospores of *Puccinia graminis*, when kept dry, can withstand considerable exposure to extremely low temperatures. This probably is true under natural conditions also, where long periods of continuous subzero centigrade temperature often prevail. The rather low temperatures of late fall are sufficient to harden urediospores and enable more of them to withstand constant subzero temperatures for long periods, but the question arises as to the effect on

the viability of urediospores of temperatures alternating above and below 0° C.

TABLE 2.—*Effect of hardening on viability of urediospores of Puccinia graminis and P. tritici* at -29° to -40° C.

Days at -29° to -40° C. (number)	Percent germination ¹ of urediospores of—							
	<i>P. graminis</i> <i>tritici</i> form 15		<i>P. graminis</i> <i>avenae</i> form 2		<i>P. graminis</i> <i>phlepratensis</i>		<i>P. tritici</i>	
	Hard- ened	Non- hard- ened	Hard- ened	Non- hard- ened	Hard- ened	Non- hard- ened	Hard- ened	Non- hard- ened
0	41.3	55.2	17.6	33.5	45.7	28.5	17.1	40.1
1	6.1	3.0	6.9	2.0	5.7	1.6	10.2	2.7
2	6.4	6.6	1.9	1.3	3.3	.3	4.8	.5
3	10.0	5.2	.9	.3	3.4	.9	.0	3.4
5	24.1	7.0	.0	.0	3.9	.0	5.8	.0
10	7.2	.2	2.1	.2	8.5	.0	0.0	3.4
15	10.4	3.0	.9	1.1	8.0	.9	.9	.0
20	8.2	.6	.6	.0	5.3	.0	.0	.0
25	4.8	.0	.0	.0	.0	.0	2.9	3.0
30	9.4	.9	1.1	.0	3.2	.3	.0	1.7
35	8.1	.03	.0	.0	1.3	.2	.0	.0
40	1.2	1.1	.6	.0	1.9	.0	1.0	.0
45	2.5	.04	.0	.0	.9	.0	.0	.0

¹ Average of 3 trials in all cases except *P. tritici*, in which there were 1 and 2 trials, respectively.

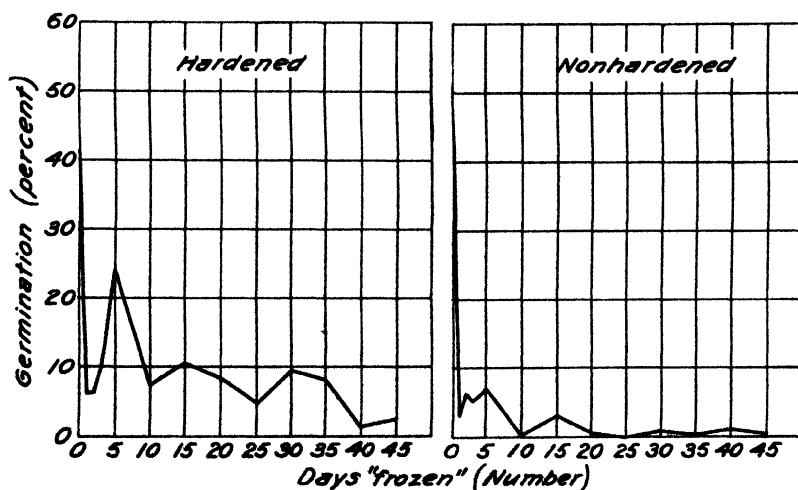


FIGURE 1.—Effect of hardening on resistance of urediospores of *Puccinia graminis tritici* form 15 to temperatures of -29° to -40° C.

EFFECT OF ALTERNATE FREEZING AND THAWING ON VIABILITY OF UREDIOSPORES

Stakman and Piemeisel (24) suggested that the reason urediospores of *Puccinia graminis tritici* do not survive the winter in Minnesota is that they are killed by "alternate freezing and thawing" in the spring. Lambert (12) also intimated that the reason urediospores survived the winter of 1923-24 at San Antonio and not at Dallas, Tex., was that alternate freezing and thawing occurred only 4 times at San Antonio and 20 times in the Dallas district.

Attempts were made to ascertain what effect temperatures alternating between -29° to -40° and $+20^{\circ}$ C. had on the viability of urediospores under dry conditions. Sets of spore-dusted slides were prepared, sufficient in number to permit germination tests at the end of 1, 2, 3, 6, 9, 12, and 15 days, respectively. The slides were kept alternately at the subzero temperature -29° to -40° for 24 hours and at 20° for 24 hours, and a control set was kept at -29° to -40° .

For the first 3 days of the experiment there was no significant difference in the survival of urediospores of *Puccinia graminis tritici* form 15 and *P. triticea* when exposed alternately to subzero and above-zero temperatures or to constant low temperatures (table 3). However, after 6 days' exposure to temperatures alternating between -29° to -40° and 20° C., spores of *P. triticea* were more severely

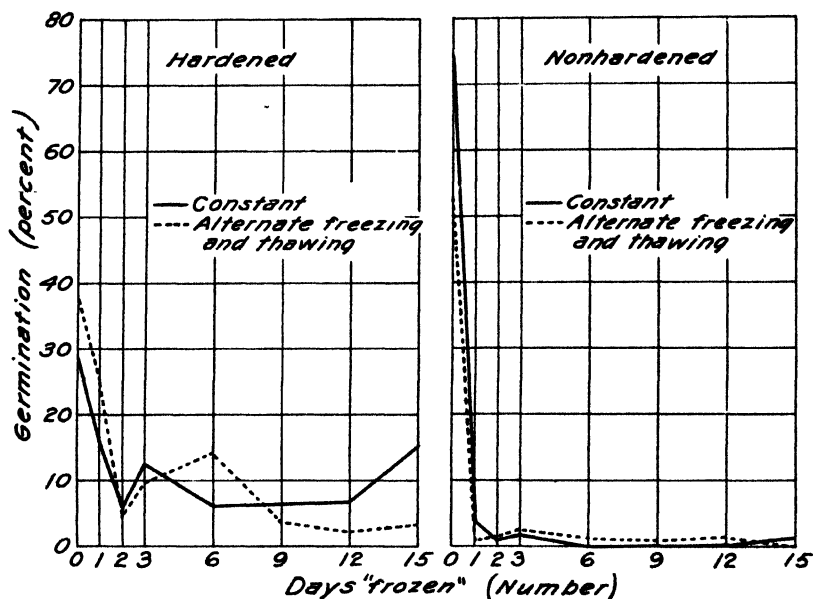


FIGURE 2.—Effect of alternating subzero and above-zero centigrade temperatures on the viability of hardened and nonhardened urediospores of *Puccinia graminis tritici* form 15.

injured than at a constant temperature of -29° to -40° . On the other hand, there appeared to be no consistent differential effect of constant and of alternating temperatures on urediospores of *P. graminis tritici*, except in the case of hardened spores after 9 days (fig. 2), when the spores withstood constant low temperature better than alternating temperatures. In all cases hardened urediospores withstood the low temperature better than nonhardened spores (fig. 2).

Of the spores of *Puccinia graminis tritici* exposed to alternating temperatures, more nonhardened than hardened spores were killed by the first exposure to a subzero temperature. After the marked diminution in percentage of germination during the first 2 days of exposure, there was no perceptible further diminution in hardened

spores until they had been exposed alternately to subzero and above-zero temperatures during a period of 9 days (fig. 2).

TABLE 3.—Effect of alternate subzero and above-zero centigrade temperatures on viability of hardened and nonhardened urediospores of *Puccinia graminis tritici* and *P. triticea*

Days at -29° to -40° C. alternating with 20° (number)	Percentage of germination ¹				Days at -29° to -40° C. (number)	Percentage of germination ¹			
	<i>P. graminis tritici</i> form 15		<i>P. triticea</i>			<i>P. graminis tritici</i> form 15		<i>P. triticea</i>	
	Hard-ened	Non-hard-ened	Hard-ened	Non-hard-ened		Hard-ened	Non-hard-ened	Hard-ened	Non-hard-ened
0	37.6	52.9	55.8	23.9	0	28.9	74.1	56.3	47.7
1	24.9	.7	42.6	3.6	1	16.0	3.8	22.4	10.9
2	4.4	1.3	9.4	2.6	2	5.9	.9	30.8	6.4
3	9.8	2.3	29.9	3.0	3	12.5	1.8	17.6	3.8
6	14.1	1.2	6.5	.4	6	6.1	.0	18.8	2.5
9	3.6	.8	7.8	.0	9	6.5	.3	32.9	11.0
12	2.1	1.3	9.1	.3	12	6.7	.3	27.7	6.6
15	3.1	.0	1.4	.1	15	15.2	1.4	22.5	1.8

¹ Average of 2 trials.

EFFECT OF TEMPERATURE ON LENGTH OF INCUBATION PERIOD

Stakman and Lambert (21, p. 374) concluded that “ * * * in the past, there has been a tendency for destructive epidemics to develop in warm growing seasons, and for cool seasons to be comparatively free from rust.” Temperature undoubtedly influences the length of the incubation period of rust and therefore influences the number of uredial generations and the consequent increase of inoculum with each successive generation. Four generations during a warm growing season may furnish very large amounts of inoculum, but with only two generations during a cool growing season the increase in inoculum would be appreciably less.

Several investigators have reported results of experiments on the effect of temperature on the incubation period. According to Stakman and Levine (22, p. 69), uredia of *Puccinia graminis* may appear 6 or 7 days after inoculation at a temperature of 66.5° to 70° F. Their results show that—

at low temperatures the development of the uredinia was retarded at the rate of 1 day for every 5 degrees of fall in temperature. Infection resulted at as low temperatures as the host could possibly stand. The spores were rather small, but the difference was not as great as in the case of high temperatures, with moderate temperature as a basis for comparison.

Mehta (16) found that uredia of *P. graminis* appeared 21 to 22 days after inoculation at a mean temperature of 40° F., in 14 to 15 days at 50.8° , in 11 to 12 days at 54.1° , and in 8 to 9 days at 66.5° . In 1923 Peltier (17, p. 49) concluded as follows regarding *P. graminis tritici* form 9:

However, plants held at 5° for 9 weeks did not develop any rust until they were transferred to a higher temperature. On the other hand, the incubation period of the rust on plants placed in the 10° , 15° , 20° , 25° , and 30° cases was 18, 16, 11, 8, and 8 days respectively. * * * Plants inoculated at 5° C. and maintained at this temperature for 7 weeks developed no rust until they were transferred to a higher temperature.

The writer made experiments to determine the effect of different temperatures on infection and on subsequent development of the rust. The effect of temperature on the entrance and the establishment of the rust in the host was determined first. Three series of plants were inoculated and placed in incubation chambers for 48 hours at 0° to 1°, 10°, and 20° C., respectively. Then, to determine the effect of temperature on the subsequent development, one-third of each set was placed in a room at 20°, another third at 10°, and the remainder at 0° to 1°. All of the plants were kept constantly in a light of about 304 foot-candles.

Puccinia graminis tritici form 35 and *P. graminis avenae* form 2 caused no infection on plants kept in moist chambers for 48 hours at 0° to 1° C. and subsequently kept at the same or at higher temperatures (table 4). On the other hand, rye plants inoculated with *P. graminis secalis* form 7 and incubated similarly at 0° to 1° produced uredia at the end of 17 days at 10° and after 10 days at 20°. It is possible, of course, that the parasite may have gained entrance into the host after the latter had been placed in the warmer temperatures, but this seems unlikely.

TABLE 4.—Time required for appearance of uredia of 3 varieties of *Puccinia graminis* at different temperatures under constant light

Incubation temperature in		Time required for first appearance of uredia of ¹			
Moist chamber (48 hours)		Constant light room	<i>P. graminis tritici</i> form 35	<i>P. graminis secalis</i> form 7	<i>P. graminis avenae</i> form 2
° C.		° C.	Days	Days	Days
20	{	0-1	70	44	50
		10	14	13	14
		20	8	8	9
10	{	0-1	—	54	—
		10	16	16	17
		20	9	8	9
0-1	{	0-1	—	—	—
		10	—	17	—
		20	—	10	—

¹ Each figure is the average of three trials.

At 10° and 20° C. the urediospores of the above-mentioned forms of *Puccinia graminis* germinate and cause infection readily. A temperature of about 20° C. seems to be the optimum for the entrance and establishment of the pathogene.

The three forms of *Puccinia graminis* mentioned above can develop and produce uredia at 0° to 1°, 10°, and 20° C., if given opportunity to gain entrance and to become established in the host at an appropriate temperature (table 4). However, more time was required for urediospores to appear at the lower temperatures. At 0° to 1°, uredia of *P. graminis tritici* form 35 did not appear until 70 days after inoculation. In another experiment, *P. graminis tritici* form 15 produced urediospores normally within 10 or 11 days at 20° and within 18 days at 10°, whereas at 0° to 1° uredia failed to appear within 80 days. This indicates that physiologic forms of *P. graminis tritici* differ in their ability to develop at temperatures just above freezing.

The results suggest that in nature the mycelium may remain dormant in infected plants during the winter if the host tissues remain alive. If this happens, urediospores may be produced as soon as the weather becomes warm; and, if there are susceptible hosts nearby, infection occurs. Several weeks of cool weather then may follow infection and the mycelium may lie dormant until the temperature again rises. Then the uredia may "miraculously" appear, furnishing early inoculum to infect young grain plants and start an early epidemic.

INFLUENCE OF TEMPERATURE ON DEVELOPMENT OF MYCELIUM WITHIN HOST TISSUE

Some field observations have been made on the survival of stem rust mycelium from one season to another. Lambert (12) reports that at Dallas, Tex., the host leaves bearing old pustules were killed by alternate freezing and thawing. Mehta (16) states the following conclusions from his observations:

It is clear that the mycelium of black rust can survive milder weather and but for the absence of the source of infection, it might appear on the cereal crops in the earlier part of spring, if weather conditions are favorable.

Obviously, the host tissue must survive the winter if the rust mycelium is to survive. The question then arises as to whether the mycelium of *Puccinia graminis* can withstand as low temperatures as the host. The following experiments accordingly were made.

Three sets of wheat differentials were inoculated with urediospores of *Puccinia graminis tritici* form 15, incubated in a moist chamber at 20° C. for 48 hours, and then placed in the 0°, 10°, and 20° chambers, respectively. This experiment was repeated three times. Uredia developed on plants placed at 10° and 20°, but at the end of 60 days none had developed at 0°. Plants in the 0° room were removed to the greenhouse at the end of 40, 50, 60, 70, 80, and 90 days, respectively. On plants kept at 0° to 1° for 80 days and then transferred to 20°, uredia appeared within 8 days after the transfer, but none appeared on plants kept at 0° to 1° for 90 days.

The mycelium of *Puccinia graminis avenae* form 2 lived for 80 days at 0° to 1° C. without producing uredia, but they appeared 4 days after the infected plants were removed to the greenhouse.

In another experiment, seedling wheat plants, inoculated with *Puccinia graminis tritici* form 15 and kept in moist chambers at 20° C. for 48 hours, were placed in the 10° room under constant light and allowed to remain there until flecks developed. Then they were removed to the 0° to 1° room, and 32 days later uredia appeared.

To determine whether the mycelium of *Puccinia graminis* can withstand as low temperatures as the host plant, seedling plants of hardy winter varieties were inoculated, kept in moist chambers for 48 hours, and then placed in the greenhouse. When flecks appeared, the plants were placed in the 0° C. chamber for at least 10 days to allow them to harden, after which different sets were kept at -12°, -10°, -7°, and -5°, respectively, for 24 hours. The same procedure was followed with plants in tillering and boot stages. After freezing, the plants again were placed at 0° for 24 hours to allow them to "thaw out" gradually. They then were removed to the greenhouse and were watched for the appearance of stem rust. Each of the experiments was repeated several times.

Uredia of *Puccinia graminis tritici* form 35 appeared on Minhardi wheat in the greenhouse about 3 days after having been subjected to -5° C. for 24 hours. In a later experiment, uredia of *Puccinia graminis tritici* form 15 appeared on Kanred and Minturki wheats in 3 and 6 days, respectively, after having been subjected to -10° for 24 hours. In this experiment low temperature seemed to affect the organism more in plants of Minturki wheat than in those of Kanred wheat, as it took the uredia 3 days longer to appear. In subsequent tests at -13° the infected leaves were killed. Uredia of *Puccinia graminis secalis* form 7 appeared on plants of Petkus rye that had been exposed to -7° for 24 hours.

It is clear that at low temperatures, the rust mycelium can remain dormant for long periods in the host, and it apparently can withstand as low a temperature (-10° C.) as the host plants. However, lower temperatures must be tried to demonstrate the latter point conclusively.

EFFECT OF TEMPERATURE ON INFECTION TYPE

Stakman and Levine (22, 23) and Levine (13, 14) have shown that environmental conditions unfavorable for the host are unfavorable also for the parasite, affecting the virulence and spore size of the latter. Waterhouse (25, p. 676) recently reached the following conclusion from his experiments:

In the case of *Puccinia graminis tritici* there was change from complete susceptibility in the hotter months to complete resistance in the cooler part of the year. The reactions on several of the differential hosts showed this change. Some physiologic forms show much more marked alterations of this nature than do others. Changes from summer to winter conditions alter the reactions shown by *Puccinia graminis avenae* on "Joanette" from complete susceptibility to complete resistance.

The writer made a study of the effect of temperature on the infection type. Even at 0° C. some rust appeared on plants that had previously been inoculated and kept for 48 hours in a moist chamber at 20° , but the uredia were very small. There was no significant difference in infection types at 10° and 20° , the only noticeable difference being a higher proportion of type 3 to type 4 at 10° . However, not only were much smaller pustules produced by several physiologic forms at 0° to 1° than at 20° , but the areas surrounding the pustules were strongly etiolated. The uredia thus developed resembled type 1 as long as the plants remained at 0° to 1° , but they changed to type 3 or type 4 when the plants were placed at 20° . For example, Little Club wheat at 20° is moderately to completely susceptible to *Puccinia graminis tritici* form 35, infection types 3 and 4 ordinarily being produced; but at 0° to 1° pustules approaching type 1 were produced 70 days after inoculation in 2 out of 3 trials (fig. 3). In a later experiment, seedlings of Little Club, inoculated on February 27 with spores of *Puccinia graminis tritici* form 35, were placed at 0° to 1° on March 6. Thirty days later uredia similar to type 1 appeared.

A set of differential varieties of wheat, inoculated with *Puccinia graminis tritici* form 15 and kept at 10° C. until flecks appeared, was placed in the 0° to 1° chamber. After 32 days, pustules of "type 1"⁵ instead of the characteristic type 3 or 4, appeared on 11 of the differential hosts (fig. 4). The first leaves of plants on which the apparent

⁵ Small pustules approaching type 1 are designated "type 1."

type 1 pustules were produced at 0° to 1° were cut off, and new leaves were allowed to develop at 20°. They then were inoculated with spores of form 15, and this time the plants were allowed to remain at 20°. Normal vigorous pustules of types 3 and 4 resulted (fig. 4).

FIGURE 3.—“Type 1” uredia produced on Little Club wheat by *Puccinia graminis tritici* form 35 at 0° to 1° C. This form normally produces uredia of types 3 and 4 on Little Club wheat at 20°.

Inoculation with spores from the type 1 pustules, produced at 0° to 1° on Vernal emmer, resulted in the development of type 4 uredia when cultured at 20°. Type 1 pustules of form 15, previously formed at 0° to 1°, developed into type 3 after the plants were removed to

20°. *Puccinia graminis secalis* form 7 and *Puccinia graminis avenae* form 5 produced normal uredia even at 0° to 1°. The type 1 uredia, therefore, represented merely the lower limit of development of rust under abnormal conditions.

DIFFERENCE IN ABILITY OF DIFFERENT PHYSIOLOGIC FORMS TO DEVELOP AT 0° TO 1° C.

Physiologic forms of *Puccinia graminis* seem to vary in their ability to develop at low temperatures. This is shown by the following experiment. On February 3, 1930, 10 pots of Little Club wheat were

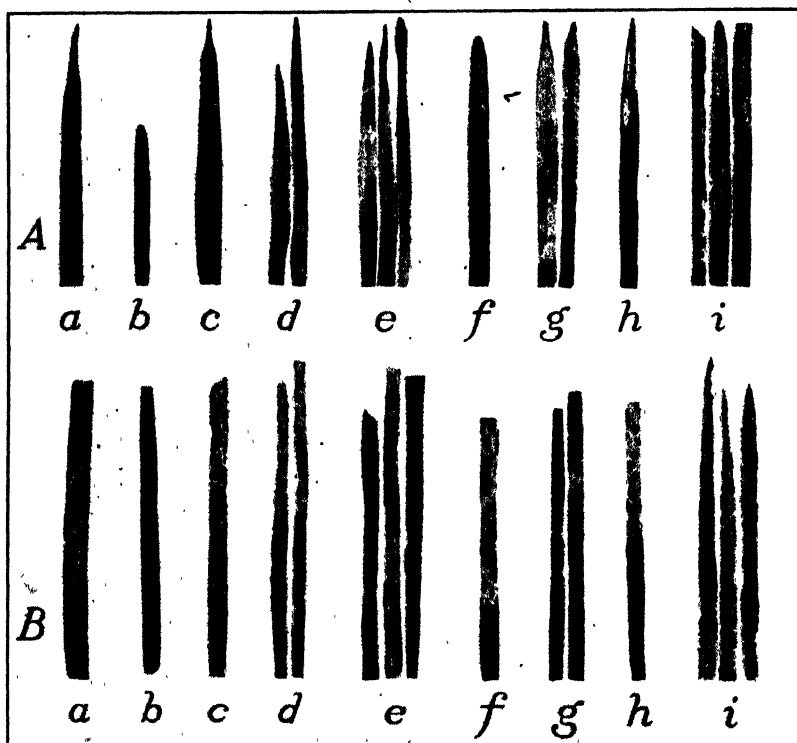


FIGURE 4.—A, Minute uredia (type 1) produced by *Puccinia graminis tritici* form 15 on varieties of *Triticum* spp. at 0° to 1° C.; B, normal development of rust at about 20° on secondary leaves of plants shown in A. a, Marquis; b, Kanred; c, Arnautka; d, Mindum; e, Spelmar; f, Kubanka; g, Acme; h, einkorn; i, Vernal emmer.

inoculated with *P. graminis tritici* form 15 and 10 with form 36. The plants were kept in moist chambers under the same conditions for 48 hours and then placed in the same greenhouse for 4 days. At the end of this period (Feb. 9) they were placed at 0° to 1° C. under constant light. Uredia of form 36 appeared on February 21, and only one uredium of form 15 had appeared by March 18, while form 36 had sporulated and produced numerous normal uredia (fig. 5). Pustules of *P. graminis tritici* form 15, however, appeared shortly after the plants again were placed in the greenhouse, showing that the mycelium

had remained alive at 0° to 1°, although there had been no appreciable development.

Two forms of *Puccinia graminis avenae* (forms 2 and 5) seemed also to develop at different rates at 0° to 1° C. For example, on February 3, 1930, several pots each of Victory oats were inoculated with these forms and kept in moist chambers under the same conditions. After 48 hours they were removed to the same greenhouse and kept 4 days. On February 9 they were placed at 0° to 1° under constant light. Uredia of form 5 appeared on February 22, while uredia of form 2 did not appear until March 3.

Evidently there are differences in the ability of physiologic forms of *Puccinia graminis tritici* and *P. graminis avenae* to develop at 0° to 1° C., whereas at 20° there are no apparent differences.

Form 7 of *Puccinia graminis secalis* developed well at 0° to 1° C., almost as well as at 10° and 20°, perhaps accounting for the prevalence of rye rust in the later fall in northern regions.

Low temperatures seem to stimulate the development of telia, because this stage of *Puccinia graminis secalis* form 7 formed abundantly

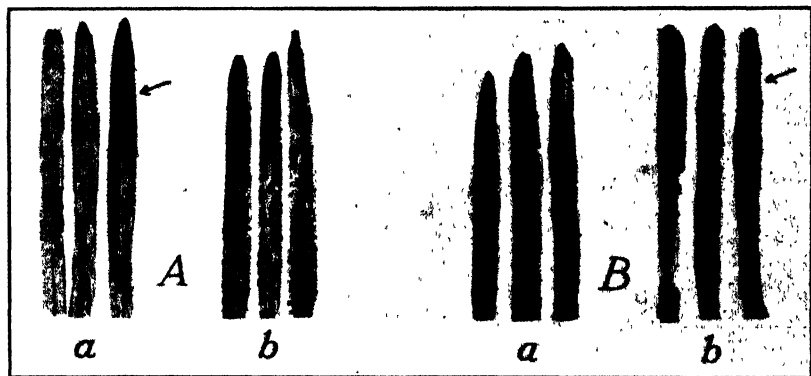


FIGURE 5.—Variation in ability of physiologic forms of *Puccinia graminis* to produce uredia at 0° to 1° C. A, Leaves of Little Club wheat seedlings infected with *P. graminis tritici*: a, Form 15; b, form 36. B, Leaves of Victory oat seedlings infected with *P. graminis avenae*: a, Form 5; b, form 2.

at 0° to 1° C. *P. graminis tritici* form 15 and form 36 produced abundant telia after the infected plants had been transferred from 0° to 1° to a warm greenhouse, where telia do not ordinarily form in abundance.

EFFECT OF LIGHT INTENSITY

The effect of light on certain rusts has been studied by several investigators. Fromme (7, p. 519) found that "Total light exclusion either early or late in the incubation period checks the development of *P. coronifera* and results in an almost complete cessation of growth." Mains (15) studied the nutritional phase and arrived at the following conclusions: "When the host is deprived of carbohydrate, *P. coronata* and *P. sorghi* require light in order to develop. However, starvation will not bring about immunity." Stakman and Piemeisel (24) pointed out that a good deal of sunlight is necessary for the best development of stem rust. They state (24, p. 487):

During periods of cloudy weather, however, the incubation period may be lengthened a week or more, and the rust does not develop so abundantly as

during bright weather. * * * The shaded plants were more weakly infected than the others. Partially etiolated plants were infected with difficulty and the rust developed very weakly on them.

Peltier (18) concluded that light is not a factor in the initial infection of wheat by stem rust, but it is essential for subsequent development. Gassner (8) also pointed out that light governs rust development in proportion to the effect it has on the metabolism of the host plant and that high light intensities tend to shorten the incubation period.

The writer made experiments with *Puccinia graminis* to determine the effect of different light intensities, at uniform temperature and humidity, on length of incubation period, type of infection, and morphology of urediospores.

Three sets of differential varieties of wheat and oats, after being inoculated and kept in moist chambers for 48 hours under uniform conditions, were placed in the 20° C. chamber under light intensities of 394, 301, and 162 foot-candles, respectively.

Under all degrees of light used the plants were much lighter in color than those grown under natural light in the greenhouse. There was slight etiolation under 394 and 301 foot-candles and considerable etiolation under 162 foot-candles. In general, oats did not thrive so well as wheat in artificial light, and plants of both cereals became very weak and etiolated after about 3 weeks. Moreover, root rot seriously hindered the growth of the plants, greater damage resulting under the light intensity of 162 foot-candles than under the higher intensities. Marquis wheat was least affected, while the durum varieties Arnautka and Spelmar were heavily infected at low light intensities. Rye thrived under artificial light for a period of about 3 weeks, after which the plants died rapidly.

EFFECT OF DIFFERENT LIGHT INTENSITIES ON LENGTH OF INCUBATION PERIOD

High light intensities shortened the incubation period appreciably. Under an intensity of 394 foot-candles, uredia of *Puccinia graminis tritici* form 15 appeared in approximately 9 days, while under the lower intensities of 301 and 162 foot-candles 10 to 11 days were required. There were no consistent differences in incubation periods in different varieties of *Triticum* spp. Uredia of *P. graminis avenae* form 2 appeared in 8 to 9 days under 394 foot-candles, while 9 to 12 days were required for their appearance under the lower light intensities.

EFFECT OF DIFFERENT LIGHT INTENSITIES ON TYPE OF UREDIA PRODUCED

A study was made of the effect of different light intensities on the infection types produced by *Puccinia graminis avenae* form 2 on 4 differential oat varieties and by *P. graminis tritici* form 15 on 11 differential wheat varieties. In addition, a comparative study was made of the effect of the same light intensities on the infection types produced by three varieties of *P. graminis*, namely, *P. graminis tritici* form 35 on Little Club wheat, *P. graminis secalis* form 7 on Petkus rye, and *P. graminis avenae* form 2 on Victory oats.

The results indicate that the different degrees of light used caused no significant or permanent changes in type of infection, although deficient light causes pustules to be considerably smaller.

From these experiments it may be concluded that under low light intensities the normal development of stem rust pustules is inhibited, resulting in the production of smaller pustules, but there is no indication of a significant or permanent change in type of infection; there is no indication that certain hosts that are susceptible under intense light are rendered resistant under low light.

EFFECT OF DIFFERENT LIGHT INTENSITIES ON SIZE AND SHAPE OF UREDIOSPORES

Urediospores produced under the various light intensities were measured and compared with those produced under sunlight at 20° C. Some similar studies had been made by Levine (13), who found that urediospores produced in deficient light were shorter than normal.

TABLE 5.—*Variants and constants for dimensions of urediospores of Puccinia graminis tritici form 15 grown under different light and temperature conditions in the greenhouse*

LENGTH					
Cultural conditions		Range of variability	Constants		
Temperature (°C.)	Light		Mean	Standard deviation	Coefficient of variability
	Foot-candles				
10	334	27.83-46.46	35.50±0.19	4.01±0.14	11.29±0.39
	394	26.91-43.47	35.19±.16	3.39±.11	9.63±.32
	301	25.53-45.08	36.13±.17	3.54±.12	9.80±.33
20	162	27.60-42.78	34.41±.16	3.42±.12	9.94±.34
	(1)	25.76-44.85	34.54±.17	3.54±.12	10.24±.35
WIDTH					
10	334	17.94-27.14	22.03±0.07	1.56±0.05	7.09±0.24
	394	18.86-27.14	22.11±.07	1.39±.05	6.27±.21
	301	19.55-27.60	21.90±.07	1.55±.11	7.07±.24
20	162	17.25-25.76	21.77±.08	1.70±.06	7.80±.26
	(1)	19.32-27.60	23.03±.07	1.52±.05	6.59±.22
RATIO OF LENGTH TO WIDTH					
		Correlation coefficient			
10	334	-0.107±0.047	1.637±0.011	0.23±0.01	14.29±0.48
	394	-.239±.045	1.621±.010	.21±.01	12.79±.43
	301	-.255±.045	1.678±.011	.22±.01	13.26±.45
20	162	-.197±.046	1.621±.011	.22±.01	13.80±.47
	(1)	-.242±.045	1.427±.010	.20±.01	14.20±.48

¹ Sunlight.

In this study the length and width of 200 spores from each of the different cultural conditions were measured at random on a slide. Because the differences were not great, it was necessary to employ statistical methods to make comparisons (tables 5 and 6). There was negative correlation between length and width of spores in all lots measured. Spores produced under 301 foot-candles were significantly longer but not significantly wider than those under 162 foot-candles, and they were significantly longer but narrower than those produced in sunlight at 20° C., and as a result of this differed

from the latter also in shape, i. e., in ratio of length to width. Spores produced under 394 foot-candles were narrower, though not significantly longer, and quite different in shape from those produced in sunlight at 20°. Those produced under 162 foot-candles were narrower than spores produced in sunlight at 20° and differed from them in shape. Spores produced under 334 foot-candles at 10° did not differ significantly from those produced under 394 foot-candles at 20°.

TABLE 6.—Differences between means of dimensions of urediospores of *Puccinia graminis tritici* form 15 cultured under different light and temperature conditions

Cultural conditions compared	Length		Width		Ratio of length to width	
	Difference in means	Difference divided by P. E.	Difference in means	Difference divided by P. E.	Difference in means	Difference divided by P. E.
334 foot-candles at 10° with 301 foot-candles at 20°	.63	2.46	.13	1.31	.041	2.66
394 foot-candles at 20° with 301 foot-candles at 20°	.94	4.01	.21	2.12	.057	3.93
394 foot-candles at 20° with 162 foot-candles at 20°	.78	3.40	.34	4.23	.000	.00
301 foot-candles at 20° with 162 foot-candles at 20°	1.72	7.35	.13	3.20	.057	3.73
394 foot-candles at 20° with sunlight at approximately 20°	.65	2.78	.92	9.29	.194	13.96
301 foot-candles at 20° with sunlight at approximately 20°	1.59	6.66	1.13	11.41	.251	17.43
162 foot-candles at 20° with sunlight at approximately 20°	.13	.56	1.26	11.85	.194	13.20

DISCUSSION AND CONCLUSIONS

The results obtained show that the death of urediospores in the winter probably is not always due to low temperatures as such. This is shown by the fact that some urediospores of the *tritici* and *phleipratensis* varieties of *Puccinia graminis* remained viable after having been exposed for 45 days to temperatures ranging from -29° to -40° C. It is true that a large percentage of urediospores usually are killed within the first 24 hours after being exposed to the low temperature, but most of those that survive retain their viability for a long time.

Hardened urediospores are more likely to survive than those not hardened. Spores produced under normal conditions were hardened at 0° C. for 10 days or longer, and their viability was compared with that of similarly produced, nonhardened spores. The hardened spores were more resistant to cold than the nonhardened. It seems probable, therefore, that spores formed at low temperatures in the fall may be more cold-resistant than those formed at higher temperatures.

Not only are some urediospores able to withstand continuous low temperatures, but a few may withstand fluctuating temperature in a surprising manner, especially when dry. It seems quite likely, therefore, that the death of urediospores in nature during the northern winters is due neither to low temperature nor to alternating low and moderate temperatures when the spores remain relatively dry, but rather to alternate freezing and thawing during wet weather.

Uredial mycelium apparently can withstand temperatures as low as those endured by the host plants. For example, mycelium remained viable within infected wheat plants kept for 24 hours at -10°C . When the plants were again placed at a temperature of approximately 20° , rust pustules developed within a few days. The mycelium within infected rye plants withstood a temperature of -7° for 24 hours and subsequently produced pustules. In nature, therefore, the mycelium probably can survive in any plant tissues that remain alive during periods of cold weather. The experiments indicate also that this mycelium can subsequently produce urediospores with the advent of favorable temperatures. This is shown by the fact that both wheat and oats infected with rust were kept for 80 days at a temperature of 0° , during which time no uredia appeared; when the plants were transferred to the greenhouse, however, uredia appeared in 8 days.

Temperature also has a profound effect on the development of rust in normal plants after infection once has taken place. For example, it required approximately a week longer for uredia to appear at 10°C . than at 20° . According to this, for every drop of 1° in temperature the incubation period would be lengthened about two-thirds of a day. This probably is not an absolute rule, as there is considerable variation in the effect of temperature on the incubation period. The generalization would be approximately true only for a range of temperatures in which the rust could develop fairly well. The fact, however, is very significant, because, in the spring wheat area of the United States, there is a relatively short time in which rust develops. Usually it is only about 45 days. At high temperatures, therefore, about 6 crops of urediospores would be produced during the season, while at low temperatures only 2 or at most 3 successive crops would be produced, and the amount of inoculum and subsequent development of rust would be greatly reduced. Field observations indicate that relatively slight differences in temperature during the critical period for the development of rust, other things being equal, determine the severity of a rust epidemic. In many years, there is a very close race between grain crops and rust. The greatest damage usually occurs just before harvest; and in many seasons the crops escape severe damage if the rust parasite is unable to produce a large amount of inoculum at that time.

Excessively low temperatures may greatly lengthen the incubation period. For example, in some experiments it required 70 days for form 35 of *Puccinia graminis tritici* to produce uredia at 0° to 1°C ., and some physiologic forms seem to be entirely unable to produce uredia at so low a temperature. It is likely that the effect of temperature may vary with the particular physiologic forms concerned in the development of rust in a given locality or in a given season.

Temperature is important not only in governing the length of the incubation period and the consequent amount of inoculum produced in a given season but also in determining the size of uredia and consequently the number of spores on each infected plant. This is clearly shown by the fact that at 0° to 1°C . *Puccinia graminis tritici* forms 15 and 35 produced pustules resembling type 1 pustules, which are extremely small, whereas these same forms produced type 4 pustules on the same variety at higher temperatures. The small pustules, however, may enlarge if the temperature rises. Tempera-

ture therefore determines the amount of inoculum in two ways: (1) by determining the number of urediospore generations in a given season and (2) by determining the number of spores per pustule.

The ability of certain physiologic forms of *Puccinia graminis* to develop at low temperatures may be important from the standpoint of regional epidemics of stem rust in the spring wheat area. If inoculum of these forms is present in this area early in the grain-growing season, stem rust can increase regardless of cool weather. This complicates the stem rust problem further, because low temperature retards the development of many forms of *Puccinia graminis tritici* sufficiently to inhibit severe infection.

As temperature extremes seem to induce early teliospore formation, teliospores are likely to be produced in nature when it is either abnormally hot or abnormally cool and when the potential danger of rust is consequently reduced. However, the effect of moisture must not be overlooked in this connection.

Light, of course, affects rust development. However, in the experiments made by the writer, the differences in light intensity were not sufficiently great to change the host reaction from susceptible to resistant or vice versa. There were fluctuations, but a type 4 infection was never reduced to a type 1 or a type 2 by withholding light. It seems unlikely that in nature there would be sufficient variation in total light during any given season to affect appreciably the development of rust.

The experiments indicate that temperature is the most important factor governing the development of rust in a susceptible host, assuming, of course, that there is sufficient moisture for infection and enough light for normal growth of plants. The stem rust pathogene seems to be well equipped to withstand vicissitudes, for it is able to persist at a very low temperature. However, its development to epidemic proportions is conditioned by temperature, as pointed out by Stakman and Lambert (21).

SUMMARY

Hardened urediospores of *Puccinia graminis* withstood subzero centigrade temperatures better than nonhardened urediospores. Hardened urediospores of wheat stem rust (*Puccinia graminis tritici*) and of timothy stem rust (*Puccinia graminis phlei-pratensis*) were able to withstand subzero temperatures for at least 45 days and hardened urediospores of oat stem rust (*Puccinia graminis avenae*) for 40 days. A few nonhardened urediospores of wheat stem rust withstood 45 days of subzero temperature; timothy stem rust, 35 days; and oat stem rust, 15 days.

Temperatures alternating above and below 0° C. had no more killing effect on nonhardened urediospores of *Puccinia graminis tritici* form 15 than did constant subzero temperatures. On the other hand, alternating temperatures killed more hardened spores after 9 days than did constant subzero temperatures.

The incubation period of stem rust is lengthened by low temperature. It took approximately a week longer for uredia to appear on plants kept at 10° C. than at 20°. Uredia developed at 0° to 1°, but much more time was required. *Puccinia graminis tritici* form 35 produced uredia in about 70 days at 0° to 1°. When the infected

plants were placed at 0° to 1° 48 hours after inoculation, form 15 produced no uredia during 80 days' exposure at this low temperature.

The mycelium of at least some physiologic forms of stem rust can withstand as low temperatures as the host. This was true of *Puccinia graminis tritici* form 35 and *Puccinia graminis secalis* form 7.

Except under extreme conditions, temperature has little effect on the type of uredia produced. At 10° C. smaller pustules were produced than at 20°. A temperature of 0° to 1° effected an apparent change of infection type of *Puccinia graminis tritici* forms 15 and 35. An infection type similar to type 1 was produced at 0° to 1° in place of type 3 or 4, normally produced at 20°. However, after the infected grain plants were taken from 0° to 1° and placed in the greenhouse at 20°, the type 1 infection developed into type 3. Therefore this temporary change at 0° to 1° was due to low temperature.

Physiologic forms of *Puccinia graminis tritici* and *Puccinia graminis avenae* differed in ability to produce uredia at low temperatures. At 0° to 1° C., *Puccinia graminis tritici* form 36 produced normal uredia readily; forms 15 and 35 produced only a few minute pustules similar to a type 1 infection; *Puccinia graminis avenae* form 2 did not produce uredia as readily as form 5. *Puccinia graminis secalis* form 7 also produced normal uredia at 0° to 1°.

A temperature of 0° to 1° C. stimulated the production of telia.

Different light intensities had no significant effect on the infection type produced, but low light intensities delayed the appearance of uredia. It took 1 to 2 days longer for uredia of *Puccinia graminis tritici* form 15 and *Puccinia graminis avenae* form 2 to appear under light intensities of 301 or 162 foot-candles than under a light intensity of 394 foot-candles.

Light intensities and quality of light seemed to affect the size and shape of urediospores. Urediospores of *Puccinia graminis tritici* form 15, formed under a light intensity of 301 foot-candles at 20° C., were significantly longer than those produced under other light conditions. Urediospores produced under artificial light were significantly longer and narrower than those produced in the greenhouse.

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A NEW CRITERION FOR THE COMPARISON OF TOXICITY WITH RESPECT TO CONCENTRATION AND TIME¹

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INTRODUCTION

In toxicological studies of rotenone and related compounds (18, 19, 20)² the method (17) used by the writer required the determination of the survival times of the test animal, the goldfish, in a number of concentrations of the toxic substance. Variables other than survival time and concentration, such as the physiological phenomenon to be used as the end point and the method of measuring it, size of the test organism, and temperature, were eliminated from consideration by restricting them to constant values in all the tests. These values were determined by practical conditions; thus, the criteria were the death point, the arithmetic average of at least 10 individuals weighing from 2.0 to 2.5 g, and a temperature of 27° C. This restriction to two variables permitted the course of toxic action under these conditions to be plotted graphically as a curve in two dimensions, and the range of concentrations used was such that the resultant curve became parallel, or nearly parallel, to both axes. Such a curve or its companion, the concentration versus reciprocal-of-survival-time curve, may serve for the derivation of a value that will express the relative toxicity of the substance, this value being obtained either from the entire curve or from certain portions of it.

CRITERIA OF OTHER WORKERS

In studying such curves a number of investigators have attempted to formulate an equation that would express the relationship between concentration and time (1 to 4, 6 to 16, 22 to 57). An equation formulated by Glaser (22) fits the data obtained by the writer better than any other. In a study of the relationship between concentration and time of action in which tadpoles were paralyzed with hydrocyanic acid, Glaser obtained a curve resembling an equilateral hyperbola. To express this relationship accurately he developed the following equation: $(C-b)(1-e^{-a(t-g)})=k$, in which C is the concentration, b is a concentration-tolerance factor (a constant), g is a time-tolerance factor (a constant), e is the base of natural logarithms, a is a proportionality factor (velocity of diffusion), t is the time, and k is a constant. The use of this equation, however, entails much experimental work as well as mathematical computation, as it requires the determination of 4 constants, 2 of which are the asymptotes of the curve.

There is still another objection to the use of Glaser's formula as a means of measuring relative toxicity. Although a value might be obtained representing the complete action of the toxic substance (as

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² Reference is made by number (italic) to Literature Cited, p. 888.

the k of an equation expressing this action), it would be difficult to interpret its significance when used as a measure of relative toxicity. It might be comprehensible if a change in toxicity were accompanied by a shift in the two tolerance factors (that is, the two asymptotes) of the same order of magnitude and direction, but this is not necessarily so. In the studies made by the writer the greatest disproportion appears in the comparison of the toxicity of nicotine (21) with that of rotenone. Compared according to the magnitude of the thresholds (the concentration-tolerance), rotenone is 800 times as toxic as nicotine, but compared according to the minimum time in which goldfish may be killed in solutions of these substances (the time tolerance) nicotine is more than 6 times as toxic as rotenone. The inadequacy of expressing relative toxicity by a single magnitude which will include the relationship of these parameters is therefore apparent.

Because the use of the entire curve involves such difficulties, for practical purposes it is desirable to find some portion of it that will suffice for a simpler comparison. In previous papers (18, 19, 20, 21) it was emphasized that there are serious objections to the comparison of the survival times at a given concentration, to the comparison of the concentrations necessary to produce death (or any other physiological phenomenon) in an arbitrary time, or to the comparison of the concentrations necessary to just cause death (threshold of toxicity concentration). If comparisons are based on the location of a point on the curve, this point should always fall on the arch of the curve, the region in which the proportional changes in the two variables, concentration and time, do not differ greatly. On either side of this region the curve flattens out so quickly that a toxic value for a point falling on either limb emphasizes one variable to the exclusion of the other; that is, on the one hand a point rapidly nears the threshold of toxicity, the lowest concentration at which the substance will have any toxic (lethal) effect, regardless of the time necessary to kill, and on the other hand a point rapidly nears the minimum time required to kill, regardless of the concentration. For these reasons the writer has used the Powers formula for the expression of relative toxicity. Powers (41) disregarded the two extremes of the curve and expressed relative toxicity by a formula dependent only on data falling within the portion of the curve approximating an equilateral hyperbola. In the concentration versus reciprocal survival-time curve (the velocity-of-fatality curve) this portion approximates a straight line (the theoretical velocity-of-fatality curve) which cuts the x axis at a point a , designated the theoretical threshold of toxicity concentration, and makes an angle, designated θ , with this axis. Thus two values are obtained, the threshold value and the slope of the theoretical velocity of fatality. Powers expressed the reciprocal relation of these factors mathematically by the equation, toxicity = $\sqrt{\frac{\tan \theta}{a}}$

In a later publication (42, p. 98) Powers states:

Perhaps a better and more simple expression for the relative toxicity is the equation of the theoretical velocity of fatality curve itself, which is the equation of a straight line. That is, $y/(x-a) = k$, where y = the velocity of fatality [the reciprocal of the survival time], x = the concentration of the solution tested, and a = the theoretical threshold of lethality concentration.

k is a constant representing the relative toxicity of the substance. The value of k is, of course, the slope of the straight line representing the theoretical velocity of fatality.

The curves obtained by the writer differed from those obtained by Powers in two respects, and therefore the use of the formula was discontinued. In the first place, Powers, using substances of low toxicity, salts of the alkali and alkaline earth metals, obtained survival-time curves that were not far removed from the x axis (the concentration axis) as compared to the y axis, whereas the writer, using highly toxic substances, obtained curves with larger and significantly different minimum times of kill. Thus, for the rotenone derivatives the Powers formula, which emphasizes two parameters, the threshold of toxicity and the maximum rate of increase of the velocity of fatality, without considering this third significant parameter, seems to be neither sufficiently inclusive nor impartially exclusive.

If this third parameter, the toxicity at high concentration (the minimum survival time or the value of the horizontal asymptote) is introduced into the formula $\sqrt{\frac{\tan \theta}{a}}$, as t_0 , it would logically appear in

the denominator—that is, the formula would read $\sqrt{\frac{\tan \theta}{at_0}}$,—and the value obtained would vary directly with the slope of the theoretical velocity-of-fatality curve and inversely with the threshold concentration and with the minimum survival time, as it should. Also, the value would now be really the square root of a square and expressible in rational units. The use of this formula for the comparison of toxicity, however, entails the determination of the asymptotes, which can be made accurately only with great difficulty.

In the second place, Powers obtained velocity curves that were sigmoid; therefore, the theoretical velocity-of-fatality curve, because it was a straight line passing through the point of inflection, was drawn with no great difficulty. However, the initial curvature, concave upwards, was not indicated in the tests with the rotenone derivatives, this possibly being due to the great toxicity of these compounds. Therefore, a straight line approximating this portion of the curve was much more difficult to locate.

The latter difficulty still obtains in the use of the slope of the theoretical velocity-of-fatality curve, that is, the equation of the straight line as suggested later by Powers (42). However, the slope would give relative toxic values based on the second parameter only, which is desirable for practical purposes. The writer is concerned here with the problem of finding the comparative toxicities of the substances at their greatest efficiencies with respect to concentration and time, that is, the range in which a quick action is commensurate with a low concentration, and not with the question of the minimum concentrations at which they just become toxic in any time no matter how great, or the minimum time it takes to kill in any concentration no matter how great. Although Powers' theoretical velocity of fatality corresponds to the portion of the survival-time curve showing this greater efficiency (the arch of the curve), in the case of rotenone and its derivatives the straight line corresponds rather to the entire

left portion of the curve to about the middle of the bend. Thus, the slope of the straight line as a criterion for the expression of relative toxicity, although nearer what is desired, is still not entirely satisfactory.

Campbell (5) suggests that the relative toxicity may be expressed numerically as an "effect comparison" by the ratio of the areas under the velocity-of-fatality curves and as a "dosage comparison" by the ratio of the areas between the curves and the y axis. Such comparisons, however, involve the use of arbitrary upper limits on the axes.

MINIMUM ct PRODUCT AS A CRITERION

Examination of the survival-time curves obtained with the rotenone derivatives with regard to the possible approximation of their equations with the asymptotic equation of the hyperbola shows two important differences. They are not asymptotic to the coordinate axes, and the ct product (the product of concentration and survival time), instead of being constant, decreases as a point moves from either extreme of a curve toward the arch and reaches a minimum value somewhere on this arch, as shown in table 1. When the curves are referred to their asymptotes as axes (that is, to c_0 and t_0 , which may be called the concentration tolerance—equivalent to Powers' a —and the time tolerance) they still are not rectangularly hyperbolic, for the product $(c-c_0)(t-t_0)$ varies in the same way as does the ct product.

TABLE 1.—Variation of the constants of the asymptotic equations of the hyperbola as shown in tests with rotenone at 27°C .

[$c_0=0.012$ mg per liter, $t_0=10$ minutes]

c	t	ct	$(c-c_0)(t-t_0)$	c	t	ct	$(c-c_0)(t-t_0)$
Milli-gram per liter	Minutes	Miligram minutes per liter	Miligram minutes per liter	Milli-gram per liter	Minutes	Miligram minutes per liter	Miligram minutes per liter
4.0	46	184	23.9	0.075	115	8.63	4.73
3.0	46	138	17.9	.055	145	7.97	4.52
2.0	48	96	15.9	.040	155	7.75	4.37
1.0	51	51	10.9	.045	172	7.74	4.36
.50	57	28.5	8.30	.040	194	7.76	4.31
.30	63	18.9	6.62	.035	250	8.75	4.83
.20	70	14.0	5.64	.025	590	14.75	7.15
.10	95	9.50	4.84	.015	2,400	36.0	7.08

It occurred to the writer that for practical purposes one of these minimum products might serve as a criterion for expressing relative toxicity. The equation $(c-c_0)(t-t_0)=k$ approximates a somewhat wider range than does the equation $ct=k$, as shown in table 1, and so might be more acceptable. To use the minimum product of $(c-c_0)(t-t_0)$, however, would entail the difficulty of determining the asymptotes. It is conceivable that more than one compound might have the same minimum ct product and yet be different in toxicity, as shown by the different positions of the arches of their survival time curves, but insofar as is indicated by the compounds studied, this difference is not likely to be great. This problem is also encountered in other arithmetic comparisons of toxicity.

The meaning of a toxic value based on the minimum ct product is disclosed by a study of the graphs in figures 1 and 2, in which are

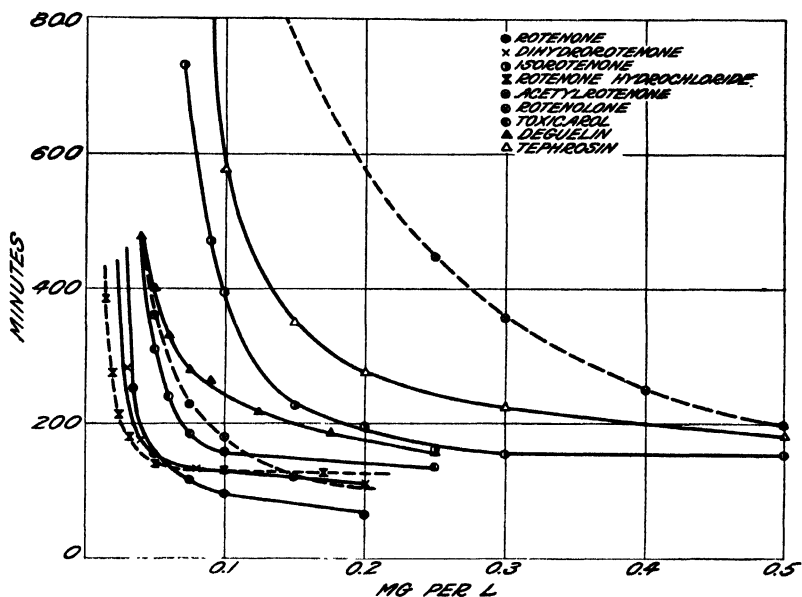


FIGURE 1.—Survival-time curves. Broken curves indicate the use of a lot of fish differing in resistance from the other lots used.

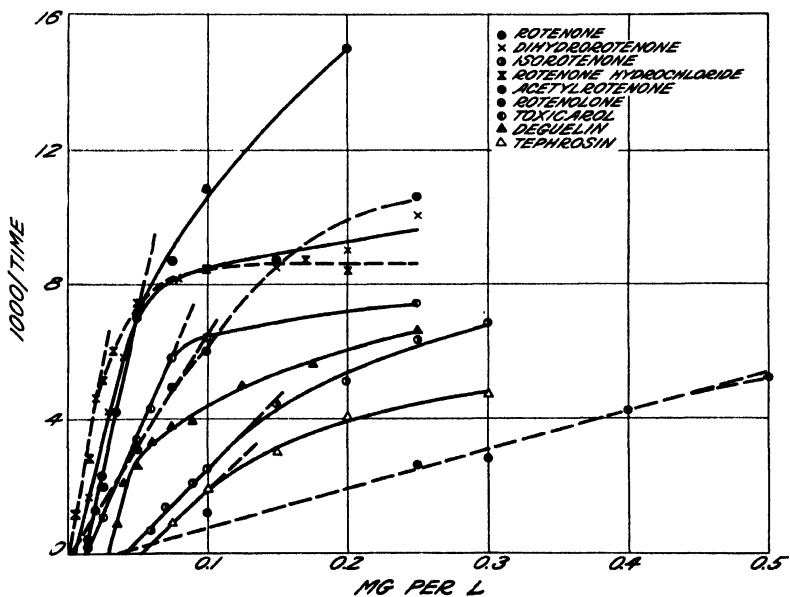


FIGURE 2.—Velocity-of-fatality curves. Broken curves indicate the use of a lot of fish differing in resistance from the other lots used.

plotted parts of the survival-time and velocity-of-fatality curves obtained with rotenone and allied compounds. In the first place, this product is the constant of the equation of an equilateral hyperbola tangent to the survival-time curve at the point corresponding to the product. The curve on either side of this point is gradually compressed within the hyperbola. Thus, it is a simple means of obtaining a hyperbola approximating the critical portion of the curve; that is, it locates a point in that region of the curve where the inverse variation of survival time with variation in concentration is most nearly proportionate. In the second place, as shown in table 2, the product is that of the coordinates of the point corresponding to the condition of greatest efficiency with regard to the two variables, concentration and time; that is, the greatest speed of toxic action is attained with the smallest quantity of toxic substance. Beyond this point, acceleration of the speed of toxic action decreases with increase in concentration, as shown in the velocity-of-fatality curves in figure 2. Thus the point is one that is medianly located with respect to the preponderant influences of the concentration tolerance factor and the time tolerance factor.

TABLE 2.—Correspondence of minimum *ct* with point of greatest efficiency

Substance	Concentration corresponding to minimum <i>ct</i>	Concentration corresponding to point of greatest efficiency	Substance	Concentration corresponding to minimum <i>ct</i>	Concentration corresponding to point of greatest efficiency
	<i>Milligram per liter</i>	<i>Milligram per liter</i>		<i>Milligram per liter</i>	<i>Milligram per liter</i>
Rotenone.....	0.045	0.050	Tephrosin.....	0.15	0.11
Dihydrorotenone.....	.040	.045	Rotenone hydrochloride.....	.023	.022
Isorotenone.....	.14	.13	Acetylrottenone.....	.075	.075
Toxicarol.....	.075	.075	Rotenolone.....	.41	.40
Deguelin.....	.055	.045			

The nearer the arch is to the origin, the greater is the toxicity of the compound; therefore, the compounds, arranged according to their toxicities as represented by this portion of the graphs in figure 1, would have the following descending order: Rotenone hydrochloride, rotenone and dihydrorotenone, toxicarol, acetylrottenone, deguelin, isorotenone, tephrosin, and rotenolone. This order is given by the comparison of the minimum *ct* products as shown in tables 3 and 4 and by none of the other methods. In addition, the apparent grouping of the compounds (fig. 1) is obviously approximated by their respective minimum *ct* products, whereas, although some of the compounds may show the same order according to another method, the quantitative comparison in these cases is not that exhibited by the graphs.

In tables 3 and 4 are given the relative toxicity data used in a comparison of the methods considered. These data were taken from papers previously published by the author (18, 19, 20).

The values given in table 4 are the relative values of the toxicities of the substances as represented by the graphs. They are not the values for the true relative toxicities; to obtain these it would be necessary to correct some of the values given for any difference in

resistance of the different lots of fish, as shown by comparative tests with rotenone. This difference was significant only in the tests with rotenone hydrochloride, acetylrotenone, and rotenolone. That the curves may be separated into two actually comparable groups is indicated by the drawing of one in solid lines and the other in broken lines. The correction to be applied will depend on the method of determining the relative toxicity. If the minimum ct product is used as the criterion, the three substances mentioned must be referred to rotenone that has given a minimum ct of 9.5 with the same lot of fish. Dihydrorotenone was also tested again with this lot. The relative value obtained, 1.4, differed somewhat from that given previously, 1.1, but since it was obtained from a more carefully controlled series of tests, it should be used. Corrected in this way, the actual relative toxicities of the substances with respect to rotenone are as follows: Rotenone hydrochloride, 1.8; dihydrorotenone, 1.4; acetylrotenone, 0.55; toxicarol, 0.55; deguelin, 0.39; isorotenone, 0.23; tephrosin, 0.15; and rotenolone, 0.097.

TABLE 3.—*Toxicity data*

Substance	c_0	t_0	$\tan \theta$	$\sqrt{\tan \theta / c_0}$	$\sqrt{\tan \theta / c_0 t_0}$	Minimum ct	Minimum $(c-c_0) (t-t_0)$
	<i>Miligram per liter</i>	<i>Minutes</i>	<i>Liter per milligram per minute</i>		<i>Liter per milligram per minute</i>	<i>Miligram minutes per liter</i>	<i>Miligram minutes per liter</i>
Rotenone	0.012	40	0.19	4.0	0.58	7.7	4.4
Dihydrorotenone	.005	75	.16	5.6	.64	7.0	3.5
Iso-rotenone	.040	145	.042	1.0	.085	33	9.3
Toxicarol	.015	75	.097	2.5	.29	14	6.6
Deguelin	.030	80	.15	2.2	.25	20	6.8
Tephrosin	.055	135	.040	.85	.073	52	20
Rotenone HCl	.002	125	.22	10	.94	5.3	
Rotenolone	.020	115	.011	.74	.069	98	51
Acetylrotenone	.002	95	.067	5.7	.59	17	

TABLE 4.—*Relative toxicity as determined by different methods*

Substance	Ratio of slopes	Ratio of values by formula 1 ¹	Ratio of values by formula 2 ²	Ratio of minimum ct	Ratio of minimum $(c-c_0) (t-t_0)$
Rotenone HCl	1.2	2.5	1.6	1.5	
Dihydrorotenone	.84	1.4	1.1	1.1	1.1
Rotenone	1.0	1.0	1.0	1.0	1.0
Toxicarol	.51	.63	.50	.55	.63
Acetylrotenone	.35	1.4	1.0	.43	
Deguelin	.79	.55	.43	.39	.56
Iso-rotenone	.28	.22	.14	.23	.53
Tephrosin	.23	.21	.13	.15	.20
Rotenolone	.058	.19	.12	.079	

¹ Powers' formula

² Suggested modification of Powers' formula.

SUMMARY

Of the equations examined, Glaser's equation, $(C-b) (1 - e^{-a(t-t_0)}) = k$, is the most nearly applicable to the concentration survival time curves obtained in studies with rotenone and related compounds in which the goldfish was the test animal. This equation, however, is too difficult to use in making a simple comparison of the toxicity of these compounds.

Powers' formula, toxicity = $\sqrt{\frac{\tan \theta}{a}}$, is unsatisfactory. A modification of this formula which includes the time-tolerance factor, $\sqrt{\frac{\tan \theta}{c_0 t_0}}$, is an improvement, but it, too, is unsatisfactory because of the necessity for determining the two tolerance factors.

The use of the slope of the theoretical velocity of fatality is unsatisfactory because it is difficult to locate accurately in some cases and because it is strongly influenced by the threshold value.

The minimum *ct* product is proposed as a criterion for comparing toxicities for the following reasons: (1) It always falls within that region of the curve in which the relation of concentration and survival time is most nearly rectangularly hyperbolic; (2) it corresponds to an intermediate condition between the predominant influence of the two tolerance factors; (3) it gives a value at the point of greatest efficiency with respect to concentration and time; and (4) it is comparatively easy to determine.

The minimum *ct* product when referred to the asymptotes as axes is unsatisfactory because of the difficulty of determining the asymptotes accurately.

The relative toxicities of the compounds studied, as determined by the minimum *ct* product, agree well with the apparent relation shown by the arching portions of their survival-time curves. This agreement is not shown by the other methods considered.

These values, referred to the value of rotenone for the same group of goldfish, are as follows: Rotenone hydrochloride, 1.8; dihydrorotenone, 1.4; acetylrotenone, 0.55; toxicarol, 0.55; deguelin, 0.39; isorotenone, 0.23; tephrosin, 0.15; and rotenolone, 0.097.

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THE QUANTITATIVE RELATIONSHIP BETWEEN THE CONSTITUTION AND TOXICITY OF SOME ROTENONE DERIVATIVES¹

By W. A. GERSDORFF

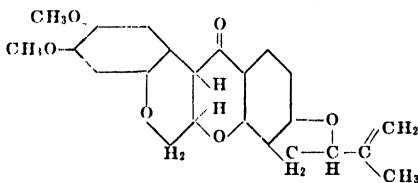
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INTRODUCTION

The preparation² of a number of derivatives of rotenone incident to the determination of its chemical structure afforded an opportunity for studying the toxicity of various highly toxic substances differing from each other but slightly in constitution. The method of studying the toxicity of an individual substance, with the goldfish as the test animal, is described in a previous paper³. From the data obtained a survival-time curve in which concentration is plotted against survival time, and a velocity-of-fatality curve in which concentration is plotted against the reciprocal of the survival time are drawn for each of the various substances. From the latter curve a value may be obtained for the maximum rate of increase of the velocity of fatality with increase in concentration. Comparisons according to this criterion, which are more serviceable from the standpoint of practicality, inasmuch as they have reference to that portion of each curve in which the proportional change in the two variables, concentration and time, is not greatly different, permit the following generalizations to be made⁴:

(1) The dihydro derivatives produced by saturation of the double bond in the side chain with hydrogen have appreciably higher toxicities than the corresponding unsaturated compounds; (2) the enol acetates and the acetyl derivatives of the hydroxy compounds have appreciably lower toxicities than the parent compounds; and (3) the hydroxy derivatives have much lower toxicities than the parent compounds.

Eight of the compounds studied may be classified in the above-mentioned groups—rotenone, dihydrorotenone, acetylrotenone⁵, rotenolone⁵, acetyldihhydrorotenone, dihydrorotenolone, acetylrotenolone, and acetyldihhydrorotenolone. The structure of rotenone⁶ is as follows:



¹ Received for publication Feb. 18, 1935, issued July 1935. Presented before the division of biological chemistry at the meeting of the American Chemical Society held at St. Petersburg, Fla., Mar. 25 to 30, 1934.

² LA FORGE, F. B., HALLER, H. L., and SMITH, L. E. THE DETERMINATION OF THE STRUCTURE OF ROTENONE. *Chem. Rev.* 12:181-213. 1933.

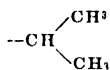
³ GERSDORFF, W. A. A METHOD FOR THE STUDY OF TOXICITY USING GOLDFISH. *Jour. Amer. Chem. Soc.* 52:3440-3445, illus. 1930.

⁴ ——— A FURTHER STUDY OF THE TOXICITY OF DERIVATIVES OF ROTENONE WITH THE GOLDFISH AS THE TEST ANIMAL. *Jour. Amer. Chem. Soc.* 56:979-980, illus. 1934.

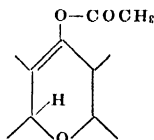
⁵ GERSDORFF, W. A. A STUDY OF THE TOXICITY OF ROTENONE HYDROCHLORIDE, ACETYLROTENONE AND ROTENOLONE USING THE GOLDFISH AS A TEST ANIMAL. *Jour. Amer. Chem. Soc.* 55:1147-1152, illus. 1933.

⁶ LA FORGE, F. B., HALLER, H. L., and SMITH, L. E. See pp. 184, 191, and 202, respectively, of reference cited in footnote 2.

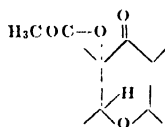
The dihydro derivatives are characterized by the saturation of the double bond in the side chain with hydrogen and so contain the group



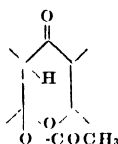
The enol acetates, acetylrotenone and acetyldihydrorotenone, which are obtained ⁶ from rotenone and dihydrorotenone on treatment with sodium acetate and acetic anhydride, are characterized by the grouping



The acetyl derivatives of the hydroxy compounds, acetylrotenolone and acetyldihydrorotenolone, which are prepared ⁶ as the main products of the reactions of iodine and an alkali acetate with alcoholic solutions of rotenone and dihydrorotenone, respectively, are characterized by the grouping



or



The hydroxy derivatives, rotenolone and dihydrorotenolone, are obtained ⁶ on saponification of the last-named acetyl compounds.

CRITERION FOR COMPARISON OF TOXICITY

Recently the author proposed ⁷ the use of the minimum product of concentration and survival time as a practical criterion for the comparison of toxicity. This value corresponds to a point at which neither tolerance factor is preponderant; it corresponds to the point of greatest efficiency with respect to concentration and survival time, and it is comparatively easy to determine.

The probable error of the observed *ct* products in the neighborhood of the minimum is no greater than 7 percent of the mean. However, the error of the given minimum value is even less, for it is determined from the survival-time curve, which is, in effect, an average of all the

⁶ See footnotes on page 893.

⁷ GERSDORFF, W. A. A NEW CRITERION FOR THE COMPARISON OF TOXICITY WITH RESPECT TO CONCENTRATION AND TIME. Jour. Agr. Research. 50:881-891, illus. 1935.

observed points. The survival-time curves are given in figure 1. Since the points corresponding to the minimum *ct* products always appear on the arches of the curves, these portions only were determined in the comparative tests made with rotenone and dihydrorotenone.

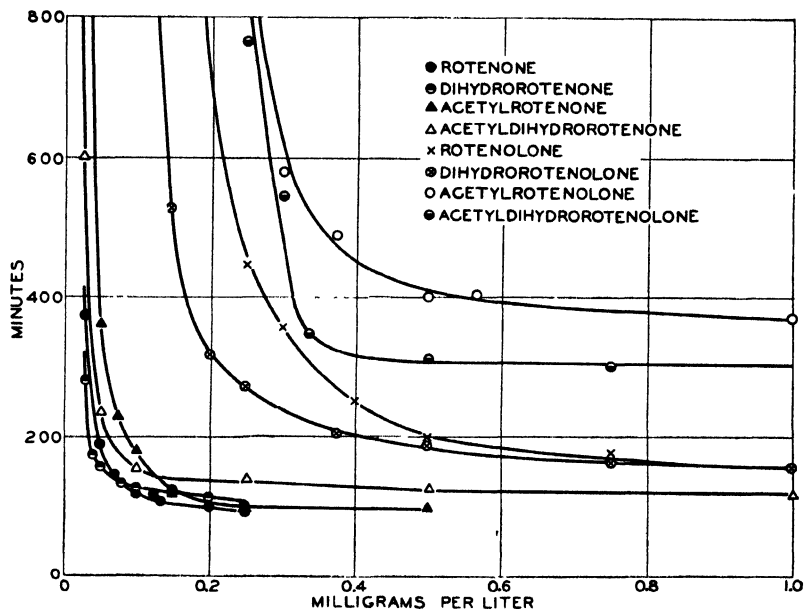


FIGURE 1 Survival-time curves.

RESULTS

Since different lots of goldfish may vary somewhat in their resistance to toxic substances and two different lots were used in the toxicological tests described in this paper, check tests were made with each lot for the purpose of comparison, rotenone being used as the standard compound. The data for these tests are given in table 1.

TABLE 1.—Toxicity of rotenone, dihydrorotenone, acetyldihydrorotenone, acetylrottenolone, dihydrorotenolone, and acetyldihydrorotenolone at 27.0° C. ± 0.2°

ROTENONE									
Concentration (mg per liter)	Fishes ¹	Mean length of fishes	Mean weight of fishes ²	Mean survival time	Concentration (mg per liter)	Fishes ¹	Mean length of fishes	Mean weight of fishes ²	Mean survival time
	Number	Mm	Grams	Minutes		Number	Mm	Grams	Minutes
0.200	³ 6	43	2.4	115	0.094	5	44	2.5	138
0.200	9	42	2.3	118	0.070	8	43	2.4	146
0.132	4	43	2.4	121	0.052	10	45	2.6	185
0.124	9	43	2.4	128	0.050	³ 7	46	2.7	190
0.100	19	44	2.5	123	0.027	³ 13	47	3.1	375
0.100	³ 7	44	2.5	130					

¹ Fishes from lot 4 were used in the dihydrorotenolone tests; fishes from lot 2 were used in the other tests, with the exceptions noted.
² Estimated from length exclusive of tail.
³ From lot 4.

TABLE 1.—*Toxicity of rotenone, dihydrorotenone, acetyldihydrorotenone, acetylrottenolone, dihydrorotenolone, and acetyldihydrorotenolone at 27.0° C. ± 0.2°—Contd.*

DIHYDROROTENONE									
Concentration (mg per liter)	Fishes	Mean length of fishes	Mean weight of fishes	Mean survival time	Concentration (mg per liter)	Fishes	Mean length of fishes	Mean weight of fishes	Mean survival time
	Number	Mm	Grams	Minutes		Number	Mm	Grams	Minutes
0.25.....	12	42	2.3	96	0.078.....	8	42	2.3	135
0.20.....	16	44	2.6	111	0.050.....	30	40	2.1	158
0.15.....	13	41	2.2	121	0.040.....	23	39	1.9	175
0.10.....	21	42	2.3	125	0.030.....	14	40	2.1	280
0.080.....	13	40	2.1	132					
ACETYLDIHYDROROTENONE									
2.00.....	8	40	2.1	122	0.100.....	³ 14	46	2.8	150
1.00.....	11	39	1.9	116	0.050.....	12	43	2.4	230
0.500.....	11	40	2.0	122	0.050.....	³ 15	47	3.1	238
0.250.....	11	41	2.2	137	0.025.....	³ 20	48	3.3	600
0.100.....	8	43	2.4	155					
ACETYLROTENOLONE									
2.00.....	10	41	2.2	⁴ 320	0.500.....	4	44	2.5	400
1.50.....	³ 12	47	3.1	365	0.375.....	³ 11	46	2.8	489
1.00.....	6	38	1.7	370	0.300.....	³ 13	48	3.2	580
0.563.....	³ 15	45	2.6	406	0.250.....	9	46	2.7	910
DIHYDROROTENOLONE									
1.00.....	10	46	2.7	155	0.200.....	12	44	2.6	319
0.750.....	12	45	2.7	161	0.143.....	11	43	2.5	530
0.500.....	12	45	2.6	188	0.102.....	12	46	2.7	⁸ 800
0.375.....	11	45	2.7	203	0.075.....	12	(⁶)	(⁶)	(⁷)
0.250.....	10	46	2.6	272					
ACETYLDIHYDROROTENOLONE									
2.00.....	7	40	2.1	280	0.500.....	³ 15	46	2.8	312
2.00.....	³ 13	46	2.9	299	0.333.....	³ 16	47	3.1	348
1.33.....	³ 12	46	2.9	312	0.300.....	³ 16	48	3.2	547
1.00.....	6	40	2.0	⁸ 344	0.250.....	9	43	2.5	767
0.750.....	³ 15	46	2.7	300	0.100.....	4	40	2.0	(⁶)
0.500.....	4	38	1.7	309					

³ From lot 4.⁴ During the initial 15 minutes of the test the temperature was too high, being 27.7° C. at the start, therefore the survival time value recorded is probably somewhat low.⁵ Owing to an increase in room temperature overnight, the temperature of the test rose to 27.9° C.; therefore the value recorded for the survival time is probably low.⁶ All fishes were not measured, but they were of the same approximate size as in the other tests.⁷ 35 hours for 9 fishes; 3 fishes were apparently unaffected after 96 hours.⁸ The temperature was too low during this test, the average being only 26.0° C. ± 0.2°. Therefore, the value recorded for the survival time is high.⁹ 17 hours for 3 fishes; 1 fish was apparently unaffected after 48 hours.

In the tests on three of the compounds both lots ⁸ were used. In the tests with dihydrorotenolone lot 4 alone was used. This lot was chosen to finish the tests because the goldfish, although of larger average size, were apparently similar to lot 2 in their resistance to these toxic substances. The results obtained with them fall well within the limit of error of the tests with lot 2. This fact is shown by the tests with all the compounds in which both lots were used. Lot 2 is from the same group of fish that was used in the previously pub-

⁸ Lot 2 was obtained in October 1931, and lot 4 was obtained in January 1933.

lished tests with rotenolone and acetylrotenone.⁹ The toxicity data for the derivatives other than these two are given in table 1.

The relative toxicity of the derivatives, obtained by comparison of their respective minimum *ct* products, designated (*ct*)_m, with that of rotenone is given in table 2. In table 3 the relation between the structure of the compounds and their toxicity is shown by the ratios between the values for the parent compounds and their derivatives. They are arranged according to the three groups, the dihydro derivatives, the acetates, and the hydroxy derivatives. There are four ratios in each group, and it is apparent that within each group they are practically identical.

TABLE 2.—Relative toxicity of rotenone derivatives at 27.0° C. ± 0.2°

Compound	Concentration	Survival time	Minimum <i>ct</i>	Relative toxicity referred to rotenone
	Milligram per liter	Minutes	Milligram minutes per liter	
Dihydrorotenone	0.035	195	6.83	1.39
Rotenone	.050	190	9.50	1.00
Acetyldihydrorotenone	.050	234	11.7	.812
Acetylrotenone	.075	229	17.2	.552
Dihydrorotenolone	.200	319	63.8	.149
Rotenolone	.410	240	98.4	.0965
Acetyldihydrorotenolone	.330	352	116	.0819
Acetylrotenolone	.370	470	174	.0546

TABLE 3.—The effect of slight changes in the constitution of rotenone compounds on their toxicity

Compound	(<i>ct</i>) _m	Effect of H ¹	Effect of Ac ²	Effect of OH ³	Effect of H and Ac	Effect of H and OH	Effect of Ac and OH	Effect of H, Ac, and OH
	Milligram minutes per liter	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Rotenone	9.50							
Dihydrorotenone	6.83	139						
Acetylrotenone	17.2		55.2					
Acetyldihydrorotenone	11.7	147	58.3		81.2			
Rotenolone	98.4			9.7				
Dihydrorotenolone	63.8	154		10.7		14.9		
Acetylrotenolone	174		56.6	9.9			5.46	
Acetyldihydrorotenolone	116	150	55.0	10.1	84.8	14.8	5.89	8.19
Average		148	56.3	10.1	83.0	14.9	5.68	
Product of individual effects					83.3	14.9	5.69	8.41

¹ 100 times the ratio of the (*ct*)_m of the unsaturated parent compound to that of the dihydro derivative.

² 100 times the ratio of the (*ct*)_m of the parent compound to that of the acetate.

³ 100 times the ratio of the (*ct*)_m of the parent compound to that of the hydroxy derivative.

SUMMARY AND CONCLUSIONS

Eight compounds, rotenone and seven of its derivatives, compared according to toxicity under conditions corresponding to the minimum value of the *ct* product at 27° C., have the following relative toxicities

⁹ GERSDORFF, W. A. See footnote 5.

to goldfish weighing from 2 to 3 g. Dihydrorotenone, 1.4; rotenone, 1.0; acetyldihydrorotenone, 0.81; acetylrotenone, 0.55; dihydrorotenolone, 0.15; rotenolone, 0.097; acetyldihydrorotenolone, 0.082; and acetylrotenolone, 0.055.

Insofar as these compounds and this method of comparison are concerned, each change in chemical constitution effects a characteristic change in toxicity independent of the effect of any other change.

The dihydro derivatives produced by saturation of the double bond in the side chain with hydrogen have 1.5 times the toxicity of the corresponding unsaturated compounds.

The acetates, whether of the enol type or the acetyl derivatives of the hydroxy compounds, have 0.56 the toxicity of the parent compounds.

The hydroxy derivatives have 0.10 the toxicity of the parent compounds.

The combined effect on toxicity of more than one change in constitution is equal to the product of the individual effects. Thus, the dihydroacetates have 0.83 the toxicity of the parent compounds; the dihydrohydroxy derivatives have 0.15 the toxicity of the parent compounds; the acetylhydroxy derivatives have 0.057 the toxicity of the parent compounds; and acetyldihydrorotenolone, the derivative including all three changes in constitution, has 0.082 the toxicity of rotenone.

EFFECT OF GOSSYPOL UPON THE MUSCLE OF THE SMALL INTESTINE OF THE RAT¹

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INTRODUCTION

Although gossypol, the toxic principle of cottonseed, has been widely studied, its physiological effect on the muscle tissue of the intestinal tract appears to have received little attention. Schwartz and Alsberg (8, p. 194)³ stated, "Gossypol has a paralytic effect not merely upon the neuromuscular apparatus of striated muscle but also upon that of smooth muscle", and in another article (7, p. 176) they reported, "The oral administration of gossypol dissolved in oil is followed by diarrhea and also by paralysis of the intestine and stomach musculature."

Edgerton and Morris (3) noted in the post-mortem examination of four rabbits which died of cottonseed poisoning that the stomach contained a large amount of food mixed with mucus and that the intestines were full. Macy and Mendel (5) made a similar observation on rabbits fed cottonseed meal. Gallup (4) noticed intestinal impaction in a rat which lived 8 days after an intraperitoneal injection of gossypol. Clark (2) found intestinal impaction in the chronic type of poisoning where rats were injected intraperitoneally. Macy and Outhouse (6) noticed that dogs fed a diet containing 45 percent of cottonseed meal for 192 days showed extreme soreness in the abdominal regions. Withers (9) noted pendulous crops in hens receiving 0.2 percent of gossypol in the diet. Thirty percent of raw cottonseed produced the same effect.

The literature cited indicates that gossypol has a paralytic effect upon the muscle tissue of the digestive tract of animals. Unpublished work by the author showed that gossypol, in oil solution, given in sufficient amounts by means of a stomach tube, caused a paralysis of the stomach musculature, and that when added directly to the bathing solution it caused paralysis of the muscle of the isolated uterus of the rat. The present work was carried out to determine the effects of gossypol upon the isolated longitudinal muscle of the small intestine of the rat.

PROCEDURE

The principal method of study was to observe the contractility of longitudinal strips of intestine after the rats had been fed gossypol or raw crushed cottonseed in various amounts for different lengths of time, the usual technic for such tissues being used. It was thought that such a procedure would more closely record the effects of gossypol than a study of the effects of gossypol added directly to the isolated

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² The author wishes to express his appreciation to Prof. B. B. Roseboom, of the physiology department of Michigan State College, for his interest in this work and for the use of apparatus in carrying out these experiments.

³ Reference is made by number (italic) to Literature Cited, p. 908.

strips of intestine. The latter method, however, was used in a few experiments, with concentrations of gossypol at 1 to 6,000, 1 to 12,500, and 1 to 25,000.

The gossypol used was prepared in the form of an acetate by Carruth's method (1). The acetic acid was removed by dissolving the gossypol acetate in ether and washing with water. The ether was driven off on a steam bath at 60° C. The crusts formed were washed with water and recrystallized from ether.

Albino and piebald rats from a healthy colony which received a ration conducive to good growth and reproduction were used. The experimental rats were generally started on the gossypol or raw crushed cottonseed diets at 30 to 40 days of age. Litter mates of the same sex and approximately of the same weight were used as controls. The experimental animal of each pair received a diet containing gossypol or raw crushed cottonseed. The control animal received the same amount of food per day without the gossypol or raw cottonseed as the experimental animal had consumed the previous day. It was thought best to follow this procedure in order to eliminate any effects which the lowered food consumption might have on the experimental animals. Except for the effect of the gossypol, the control animals would approximate the weight and nutritive state of the experimental animals at the time of slaughter. All animals had access to food up to the time they were killed.

The following basal diet was used:

	<i>Percent</i>
Ground yellow corn.....	54
Wheat middlings.....	23
Refined cottonseed oil.....	7
Alfalfa leaf meal.....	4
Protein mixture.....	9
Calcium carbonate.....	1
Salt.....	1
Bonemeal.....	1
	100

The protein mixture contained:

Tankage.....	50
Linseed meal.....	35
Crude casein.....	15
	100

When gossypol was added to the diet, the desired amount was dissolved in ether and mixed with the cottonseed oil used in the ration. This solution was poured over the dry feed mixture, which was then spread in a thin layer and allowed to remain at room temperature until the ether had evaporated. Where raw crushed cottonseed was fed it replaced the wheat middlings in the basal diet.

After the animals had been on the diets the required length of time they were removed from the cages and killed by a blow on the head. The abdomen was opened and strips about 2 cm in length were removed and placed in Locke's solution without glucose. The strips were taken from three different portions of the intestine. Most of them were taken from 12 to 25 cm below the pylorus. Others were taken from 3 to 5 cm below the pylorus and from 3 to 5 cm above the caecum. The strips were removed as needed from Locke's solution without glucose, and kymograph records were made of

their longitudinal activity in the complete Locke's solution. Oxygen was bubbled through the Locke's solution with the temperature kept constant at 37.5° C. Contractility records were made simultaneously on segments from the control and experimental animals.

The levers used measured 19 cm from the writing point to the fulcrum and 3.5 cm from the fulcrum to the thread attached to the intestine, and were so weighted as to place a stress of about 0.5 g on the strip.

In order to simplify the description and recording of the results of the contractility studies a grading system was used. Four plus means that a strip had consistently made excursions in height of contraction comparable with that which a beating strip would make

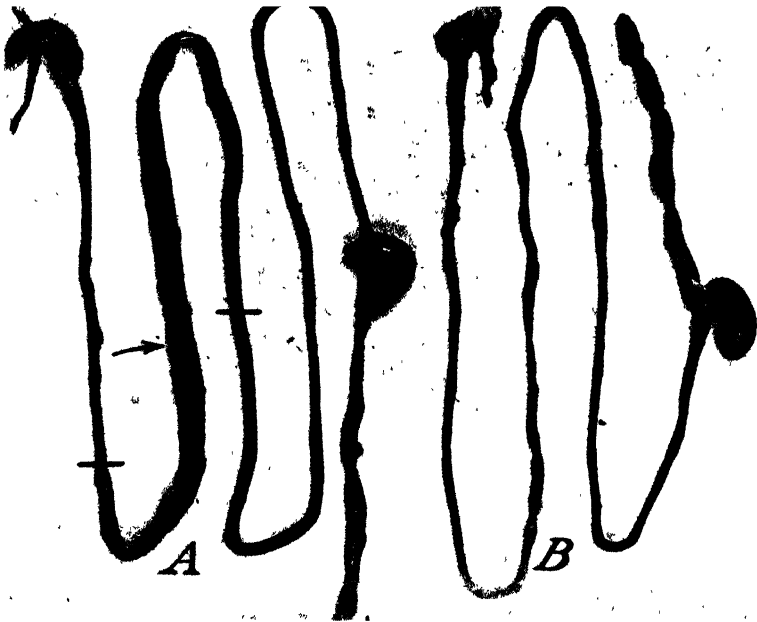


FIGURE 1. —Dilated intestine of a rat fed 17 percent of raw cottonseed for 8 weeks (A) compared with that of the control animal fed the same kind and amount of food without the raw cottonseed (B). The dilation was graded +++ to +++++.

which was normal for the weight of the animal and the particular place in the intestine from which it was taken. Zero means that the strip showed little or no activity; 1+, 2+, and 3+ represent intermediate values, and 5+ or 6+ means that the height of the excursions was greater than normal.

During the progress of the experiments it was noticed that many of the experimental animals which had received gossypol or raw cottonseed for 3 to 8 weeks in amounts that would limit growth, showed a dilation of a part of the small intestine, as indicated in figure 1. These distended or dilated intestines were graded as +, ++, +++, and ++++ according to the severity of the distension.

It was noticed during the progress of the experiments that strips of intestines from the experimental animals when placed in Locke's

solution without glucose did not shorten as much as similar strips taken from the control animals. Accordingly 6 cm strips were taken 12 to 25 cm below the pylorus from each pair of animals and placed in Locke's solution without glucose. Approximately 1 hour later they were removed, measured again, and the length recorded. Inasmuch as the strips from the various pairs were kept at different temperatures, only the strips from each pair are comparable.

Kymograph records were not made of pairs in which the experimental animals appeared to be within a few days of death.

In the tables the letter E before the numbers of an animal designates the experimental animal and the letter C designates the control.

EXPERIMENTAL RESULTS

EFFECT OF ADDING GOSSYPOL DIRECTLY TO THE SOLUTION BATHING A NORMAL INTESTINE

To determine the effect of adding gossypol directly to a solution bathing a normal intestine, two strips of intestine were taken from the same animal 12 to 25 cm below the pylorus, and one was placed in a solution to which gossypol was added at a concentration of 1 to

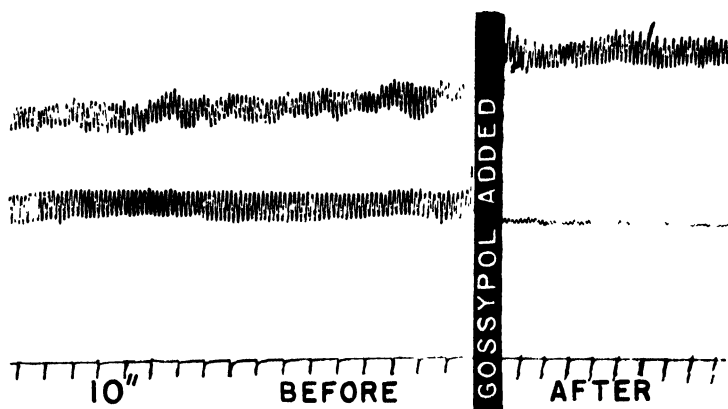


FIGURE 2 — Effect on isolated longitudinal strips of the intestine of a rat before, and 13 minutes after, adding gossypol directly to the bathing solution at a concentration of 1 to 6,000 $10'' = 10$ seconds, time interval

6,000. The other strip served as a control. The results, presented as kymograph records in figure 2, show that at a concentration of 1 to 6,000 the contractions were greatly reduced after 13 minutes. At a concentration of 1 to 25,000, as shown by other experiments, the contractions were greatly reduced after 20 minutes.

Apparently gossypol when added directly to the bathing solution caused a progressive paralysis of the muscle of the small intestine.

EFFECT OF FEEDING FROM 0.2 TO 0.4 PERCENT OF GOSSYPOL FOR 5 TO 21 DAYS ON SEGMENTS OF INTESTINE TAKEN FROM 12 TO 25 CM BELOW THE PYLORUS

By reference to table 1 it will be noted that three animals fed 0.2 percent gossypol for 17 days showed normal contraction. In these cases, however, the 6-cm strip did not shorten as much as that of the controls. In none of these three animals was the intestine dilated.

TABLE 1.—*Effect of feeding rats from 0.2 to 0.4 percent of gossypol, from 5 to 21 days, upon the contractile strength of strips of their intestines taken from 12 to 25 cm below the pylorus*

Animal no. and sex ¹	Weight when placed on experiment	Weight when killed	Gossypol-fed	Feeding period	Dilation of intestine ²	Strips used	Length of 6-cm strip 1 hour after removal	Strength of contraction
	Grams	Grams	Percent	Days		Number		Centimeters
E 541, male	71	88	0.2	17	0	2	4.0	4+
C 540, male	72	86			0	2	3.5	4+
E 547, male	83	91	.2	17	0	2	4.2	4+
C 546, male	92	97			0	2	4.0	3+
E 553, male	101	105	.2	17	0	2	3.5	4+
C 552, male	101	97			0	2	3.4	4+
E 443, male	63	60	.3	5	0	1	3.5	0+
C 444, male	64	55			0	1	3.3	5+
E 437, male	64	55	.3	7	0	2	3.4	0+
C 438, male	69	65			0	2	3.0	5+
E 439, female	102	81	.3	14	++	2	4.1	0+
C 440, female	103	92			0	2	3.3	3+
E 141, female	58	51	.3	14	+	2	4.3	0+
C 442, female	67	52			0	2	3.3	4+
E 448, male	66	49	.3	18	++	2	4.0	5+
C 447, male	65	67			0	2	3.5	4+
E 418, female	62	73	.2 .25 .3	7 7 7	++++	1	4.4	5+
C 417, female	58	69			0	1	3.4	4+
E 480, male	61	43	.4	7	0	2	4.2	6+
C 479, male	61	49			0	2	3.5	4+
E 484, female	64	46	.4	8	0	2	4.5	6+
C 483, female	63	54			0	2	3.3	4+
E 486, female	45	45	.4	10	0	2	3.4	5+
C 485, female	65	40			0	2	2.8	3+
E 488, female	69	39	.4	9	Died			
C 487, female	67	49						
E 490, male	56	38	.4	11	Died			
C 489, male	56	35						
E 482, female	61	38	.4	12	Died			
C 481, female	61	36						

¹ E, experimental, and C, control animals in all tables

² See text for explanation of symbols in this and following tables.

Of five animals fed 0.3 percent of gossypol for from 5 to 18 days, all showed an increased height of contraction as compared with the controls. In all cases the 6-cm strip from the experimental animal shortened less than normal. The tracings of experimental animal E 439 and control animal C 440 are presented in figure 3.

One animal (E 418) fed 0.2 percent of gossypol for 7 days, 0.25 percent for 7 days, and then 0.3 percent for 7 days more showed an increased height of contraction as compared to the control. This animal showed a ++++ distension of the intestine.

Three animals fed 0.4 percent of gossypol for 7 to 10 days showed increased contraction as compared to the controls. Three other animals at this level of gossypol died in from 9 to 12 days.

It would appear from this series of observations that gossypol fed at levels of 0.3 to 0.4 percent for short periods causes an increase in the height of contraction of longitudinal strips of the intestine. The 6-cm strips, however, shortened less than those of the controls and four animals of the group showed a distended intestine.

EFFECT OF FEEDING FROM 0.2 TO 0.3 PERCENT OF GOSSYPOL FOR 21 TO 42 DAYS

A series of experiments was carried out to determine the effects of feeding 0.2 to 0.3 percent of gossypol for 21 to 42 days. The tracing in figure 4, shows an almost complete paralysis of the segment of intestine of rat E 502, after the animal had received 0.3 percent of gossypol for 2 weeks, 0.2 percent for 2 weeks, and 0.25 percent for 3

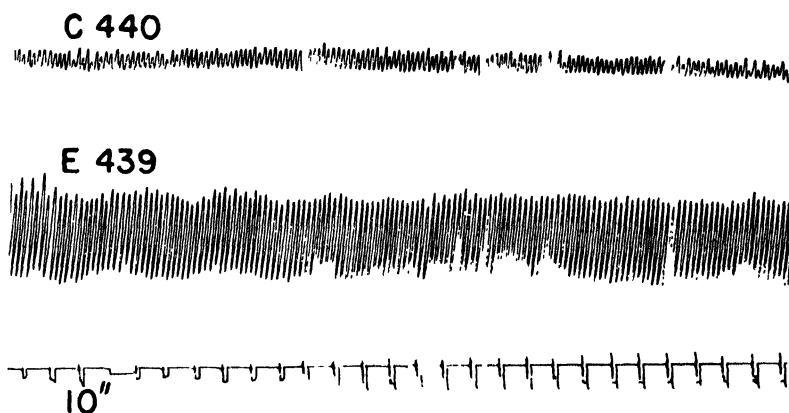


FIGURE 3.—Showing increased activity of longitudinal strip of intestine of rat E 439 fed 0.3 percent gossypol for 14 days compared to control animal C 440. 10" = 10 seconds time interval

weeks. This result was characteristic of those obtained in this series of experiments. The 6-cm strip of the intestines of the experimental animals contracted but very little as compared with that of the controls (table 2).

An experiment in which five animals were fed 0.15 percent of gossypol for 10 days and 0.3 percent for 32 days, but in which the food consumption of the controls was not limited, gave the same results.

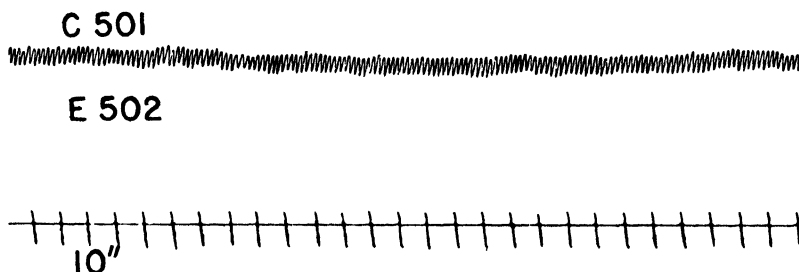


FIGURE 4.—Almost complete paralysis of the segment of intestine of rat E 502 fed 0.3 percent gossypol for 2 weeks, 0.2 percent for 2 weeks, and 0.25 percent for 3 weeks as compared with control animal C 501. 10" = 10 seconds time interval

A comparison of the results shown in table 1 with those in table 2 suggests that a large amount of gossypol fed for a short period causes a stimulation of the longitudinal strips and that the feeding of smaller amounts for longer periods finally causes a paralysis of the strips.

TABLE 2.—*Effect of feeding rats from 0.2 to 0.3 percent gossypol, from 21 to 42 days, upon the contractile strength of strips of their intestines taken from 12 to 25 cm below the pylorus*

Animal no. and sex	Weight when placed on experiment	Weight when killed	Gossypol fed	Feeding period	Dilation of intestine	Strips used	Length of 6-cm segment 1 hour after removal	Strength of contraction
	Grams	Grams	Percent	Days		Number	Centimeters	
E 504, female.	80	58	$\begin{Bmatrix} 0.3 \\ .25 \end{Bmatrix}$	$\begin{Bmatrix} 7 \\ 35 \end{Bmatrix}$	+	1	5.5	1+
C 503, female.	75	72			0	1	3.5	3+
E 506, male.	83	61	$\begin{Bmatrix} .3 \\ .25 \end{Bmatrix}$	$\begin{Bmatrix} 7 \\ 35 \end{Bmatrix}$	+	1	5.5	1+
C 505, male.	73	76			0	1	3.5	4+
E 510, female.	83	70	$\begin{Bmatrix} .3 \\ .25 \end{Bmatrix}$	$\begin{Bmatrix} 7 \\ 35 \end{Bmatrix}$	0	1	5.5	1+
C 509, female.	75	91			0	1	3.5	4+
E 508, male.	73	58	$\begin{Bmatrix} .3 \\ .25 \end{Bmatrix}$	$\begin{Bmatrix} 7 \\ 35 \end{Bmatrix}$	++++		(1)	-----
C 507, male.	73	95						
E 498, male.	100	106	$\begin{Bmatrix} .3 \\ .25 \end{Bmatrix}$	$\begin{Bmatrix} 14 \\ 12 \end{Bmatrix}$	+	1	5.7	2+
C 497, male.	88	175			0	1	3.0	3+
E 500, male.	88	79	$\begin{Bmatrix} .3 \\ .25 \end{Bmatrix}$	$\begin{Bmatrix} 14 \\ 12 \end{Bmatrix}$	++++	1	5.5	1+
C 499, male.	86	100			0	1	3.1	4+
E 502, male.	87	92	$\begin{Bmatrix} .3 \\ .25 \end{Bmatrix}$	$\begin{Bmatrix} 14 \\ 12 \end{Bmatrix}$	+	1	5.5	1+
C 501, male.	87	100			0	1	3.7	4+
E 408, male.	62	71	$\begin{Bmatrix} .25 \\ .3 \end{Bmatrix}$	$\begin{Bmatrix} 7 \\ 7 \end{Bmatrix}$	+++	2	5.1	1+
C 407, male.	64	74			0	2	3.0	4+
E 416, female.	73	63	$\begin{Bmatrix} .25 \\ .3 \end{Bmatrix}$	$\begin{Bmatrix} 7 \\ 4 \end{Bmatrix}$	++	2	4.8	1+
C 415, female.	67	71			0	2	3.0	4+

1 Almost dead.

EFFECT OF FEEDING 17 PERCENT OF RAW COTTONSEED FOR 42 TO 56 DAYS ON SEGMENTS OF INTESTINE TAKEN FROM 12 TO 25 CM BELOW THE PYLORUS

Although the experiments thus far reported were carried out with gossypol directly, in most of the preliminary work raw cottonseed was used as the source of gossypol. The results with three pairs of animals are shown in table 3. The effects were much the same as when gossypol was fed.

TABLE 3.—*Effect of feeding rats 17 percent raw cottonseed, from 42 to 56 days, upon the contractile strength of strips of their intestines taken from 12 to 25 cm below the pylorus*

Animal no. and sex	Weight when placed on experiment	Weight when killed	Feeding period	Dilation of intestine	Strips used	Length of 6-cm segment 1 hour after removal	Strength of contraction
	Grams	Grams	Days		Number	Centimeters	
E 512, male.	71	80	42	++	2	5.6	0
C 511, male.	75	100		0	2	3.5	4+
E 516, female.	80	75	42	+++	2	5.3	2+
C 515, female.	74	85		0	2	3.5	3+
E 514, male.	71	100	56	+	1	5.8	0
C 513, male.	65	117		0	1	3.7	3+

In another series of experiments four animals fed 17 percent of raw cottonseed for 5 weeks were killed and the segments from three different portions of the small intestine were compared directly with each other. The two segments taken from the upper part of the digestive tract showed marked paralysis, while those taken from 3 to 5 cm above the caecum were but slightly affected.

TABLE 4.—Comparative effect of feeding gossypol to rats upon the contractile strength of strips from different parts of their intestines

Animal no. and sex	Strength of segment 3-5 cm below the pylorus	Strength of segment 12-25 cm below the pylorus ¹	Strength of segment 3-5 cm above the caecum
E 504, female.....	0	1+	3+
C 503, female.....	2+	3+	1+
E 506, male.....	0	1+	4+
C 505, male.....	4+	4+	4+
E 510, female.....	1+	1+	3+
C 509, female.....	2+	4+	3+
E 500, male.....	0	1+	2+
C 499, male.....	4+	4+	3+
E 502, male.....	0	1+	4+
C 501, male.....	4+	4+	3+

¹ Data taken from table 2.

EFFECT OF GOSSYPOL ON SEGMENTS FROM OTHER REGIONS OF THE SMALL INTESTINE

In the experiments thus far presented all the segments studied were taken from 12 to 25 cm below the pylorus. This region was selected because it was here that the effect of the gossypol seemed to be most pronounced as judged by the fact that at this place a dilation of the intestine usually occurred.

From animals E 504, E 506, E 510, E 500, and E 502 (table 2) two strips were taken, one from 3 to 5 cm below the pylorus and the other from 3 to 5 cm above the caecum, and compared with similar strips from the controls. The results are shown in table 4.

PECULIAR RHYTHM IN DILATED SEGMENTS

A peculiar rhythm was noticed in eight segments from intestines which were greatly dilated—a number sufficiently large to be of some significance. This rhythm is shown in figure 5. The length of time between these peculiar reactions as well as the strength of contraction varied, of course, with different animals. This rhythm was never noticed except in the dilated portions of the intestine. In three instances it was duplicated in a second strip from the same animal.

DISCUSSION OF RESULTS

To conserve space, only the results from typical series of animals have been reported. The results of the investigation indicate that when gossypol is fed to rats in large amounts for 5 to 21 days, it causes a stimulation of the isolated longitudinal muscle of the small intestine, as shown by a study of strips taken from 12 to 25 cm below the pylorus. This effect may in part explain why investigators in the past have noticed diarrhea after gossypol or raw cottonseed has been fed to

animals. It may be that the increased stimulation of the intestine was an attempt of the animal to get rid of the toxic material. This stimulation was not apparent when gossypol was added directly to the isolated tissue in the bathing solution. It would seem, therefore, that the method used in this work more closely records the true physiological effects of a drug than the method of adding the material directly to the bathing solution.

The results further indicate that when the animals survive a medium dosage of gossypol for 4 to 8 weeks, a paralysis of the isolated longitudinal muscle takes place in the region about 12 to 25 cm below the pylorus. These animals would be considered by most investigators as in a chronic state of injury. No explanation of the mechanism by which the paralysis is produced will be attempted. A considerable number of segments of the various animals were subjected to pilocarpine in dilutions of 1 to 50,000. The results, however, were not consistent except in cases where the strips showed almost complete paralysis. In these cases the strips did not react to pilocarpine.

Since large amounts of gossypol fed for short periods stimulated the contractility of the muscle of the intestine in strips taken from 12 to

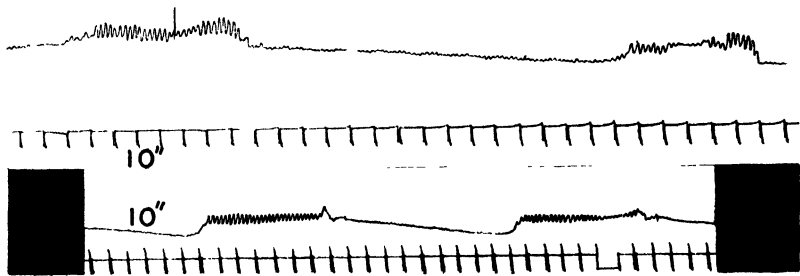


FIGURE 5 Peculiar rhythm in strip of intestine taken from two different rats with a dilated segment of intestine 10" = 10 seconds time interval

25 cm below the pylorus and small amounts fed for long periods produced paralysis, it might be expected that the feeding of intermediate amounts for intermediate lengths of time would produce results in which some strips would show stimulation, some paralysis, and some would appear normal. This was the case in several series of experiments not reported in this paper.

Observations on different portions of the small intestine indicated that the lower portion of the small intestine was not markedly affected in animals in a chronic state of injury. Segments taken from 3 to 5 cm above the caecum contracted in about a normal manner.

During the progress of this experiment it was noticed that strips of intestine from animals fed gossypol became fatigued more easily than those from the controls. When animals had been fed gossypol for a considerable length of time, and records were made of strips taken from the same part of the intestine, it was found that if the first strip showed some activity, the second showed less and the third still less. In the case of the controls, however, the records showed no such decrease in activity.

Dilation of the intestine occurred in 22 of the animals reported in this investigation and in 33 of those not reported. The dilation could

be produced either with gossypol or raw cottonseed with equal regularity. It occurred most frequently in the animals that showed slow growth during the time they received the gossypol or raw cottonseed. It always occurred in the region indicated by an arrow in figure 1. In some cases the dilated segment of intestine was 7 or 8 times the normal size. Histological sections of the enlarged part on did not show any particular alteration.

In most cases the strips of intestine from the animals fed gossypol lacked the ability to shorten, although in certain instances they displayed some contractility. This observation is of some importance in this problem, for it seems to indicate that the musculature of the intestine had been affected in some way.

Although no kymograph records were made of the circular muscle of the small intestine, it might be expected that this muscle would be affected in the same manner as the longitudinal muscle since histologically it is the same type of muscle. The fact that segments of the intestines of the animals fed gossypol were dilated lends support to this view.

The evidence indicates that gossypol has a definite effect on the neuromuscular system of the upper portion of the small intestine of the rat, as is shown by the lowered strength of contraction, dilation of that portion of the intestine, and lack of ability of the segments to shorten.

It cannot definitely be stated whether the absence of effect on the lower portion is due to the fact that the gossypol has been digested and detoxified in the upper portion of the intestine and therefore does not have a chance to act on the lower portion, or whether gossypol acts specifically on certain portions of the tract.

No explanation is offered for the peculiar rhythm noticed in a few of the intestines which were greatly dilated. This type of contraction was never observed in any part of the intestine except the dilated part.

SUMMARY

Gossypol fed to rats at a 0.3- to 0.4-percent level for 5 to 21 days caused a stimulation of the isolated longitudinal muscle of the small intestine in segments taken from 12 to 25 cm below the pylorus.

Gossypol fed to rats at a 0.2- to 0.3-percent level for periods greater than 3 weeks and up to 8 weeks caused a partial paralysis of the isolated longitudinal muscle of the small intestine in segments taken from 12 to 25 cm below the pylorus.

In a limited number of rats in a state of chronic injury segments taken from 3 to 5 cm above the caecum were not markedly affected.

Gossypol fed at levels that permitted little growth usually caused a dilation in the upper portion of the small intestine of the rat.

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A STUDY OF THE PALATABILITY AND POSSIBLE TOXICITY OF 11 SPECIES OF CROTALARIA, ESPECIALLY OF *C. SPECTABILIS* ROTH¹

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INTRODUCTION

Various species of the genus *Crotalaria* have been found to be adapted culturally to the lighter sandy soils of the Coastal Plain (5)³ and are comparable in composition at early stages of growth with other legumes used in feeding livestock. Since within the genus, certain species perhaps have value as feeds for livestock, a joint study was undertaken by the United States Department of Agriculture and the Florida Agricultural Experiment Station, to locate and investigate such promising species. An investigation was undertaken to compare, by means of grazing and feeding trials, the relative palatability of the green forage, the artificially dried hays, and the silages made from several species. Tests were made in 1931, 1932, and 1933. Indications of the toxicity of one species—*C. spectabilis* Roth—to cattle were observed among the animals used, as was stated in a previous publication (7, footnote, p. 621). In December 1931 Thomas encountered field cases of toxicity of the seeds of this species to chickens, and studied more closely in the laboratory the reactions of chickens, turkeys, quail, and doves to these seeds (9). The toxic principle was isolated by Neal and Rusoff (6), who described it and studied certain of its properties. Emmel, Sanders, and Henley (4) recently found ground seeds of *C. spectabilis* to be toxic to swine.

Four species of *Crotalaria* are known to be toxic to livestock. Symptoms and lesions differ, indicating that the toxic principles are not the same in all species. Bessey and Stalker (2) showed *C. sagittalis* L. to cause "Missouri River bottom disease", which resulted in death among horses. Seeds of *C. juncea* L., or sunn-hemp (1) were fed to a healthy mature sheep in amounts of one-fourth pound daily for 14 days, and one-half pound for 12 additional days. Weakness, a tucked-up appearance, and catarrh developed shortly after the fourteenth day. Death occurred on the twenty-sixth day. According to Burt-Davy (3), cattle fed *C. burkeana* Benth for 5 days became stiff in the joints, moved slowly, and were unable to stand ultimately. The hoofs lengthened and broke, and death resulted from starvation.

¹ Received for publication Feb. 11, 1935; issued July.

² This study is an outgrowth of a cooperative investigation of the feeding value of crotalaris conducted jointly by the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Departments of Agronomy and Animal Husbandry, Florida Agricultural Experiment Station. Feeding trials were conducted by the Department of Animal Husbandry, with forages grown and provided by the Agronomy Department and by the Division of Forage Crops and Diseases, U. S. Department of Agriculture. G. E. Ritchey was in charge locally for the Division of Forage Crops and Diseases.

³ Reference is made by number (italics) to Literature Cited, p. 922.

Theiler (8) fed as much as 46 pounds of *C. dura* Wood and Evans to horses in 23 days and observed symptoms of poisoning within 16 to 80 days. The main symptoms and lesions included fever, acute polypnoea, and subsequent dyspnea, destruction of the respiratory epithelium, leading to a vicarious emphysema, granulation tissue, and degeneration of the bronchii. A bull fed 2 pounds daily died in 64 days, and an ox was killed in extremis on the ninety-eighth day. Symptoms included complete loss of appetite, diarrhea with dark-colored feces, progressive weakness, and ultimate loss of animation. Autopsy revealed cirrhosis of the liver, thickened central veins, and fibrillar bundles which formed a lacework in which blood collected.

Crotalaria striata D. C. and *C. incana* L. have been indicated as possibly toxic to cattle, sheep, and goats in certain countries, but have not been observed to be injurious in the United States.

PLAN OF INVESTIGATION

Nine species of *Crotalaria* were planted in adjacent rows in a fenced 2-acre field in 1931 and in 1932. From time to time during the grazing season, two head of cattle were transferred to this field for a brief tour, usually 14 days and the extent of grazing on the separate species was estimated daily by two or more members of the staff. No supplementary feeds were allowed. Water was accessible. Also, some volunteer grasses and other plants, not killed by routine cultivation of the field, were grazed.

Eight of the eleven species of *Crotalaria*, as listed in table 1, were harvested, artificially dried as hay, and placed in adjacent compartments of an overhead rack in a small lot, where cattle were given free access to them. Observations were made daily by two or more staff members as to the order of preference and relative amounts of each species eaten by the cattle. These observations, as well as the growth habits of the plants, were used as a basis for selection of the most promising species for further study as a forage crop. During the course of these studies, indications of toxicity of one species were obtained upon the death of three experimental animals. An additional controlled-feeding trial and careful laboratory analyses were made to prove the definite toxicity of the species *C. spectabilis*. Pertinent details relative to the toxicity of this species are outlined in this paper.

OBSERVATIONS OF CATTLE

Two cows were used in each tour on the *crotalaria* grazing plots in 1931 and 1932, the several species employed being listed in table 1. Four cows and two pigs were employed in this manner during the first season. The pigs grazed none of the *Crotalaria* during the 15-day tour. During the last 4 days of the fourth tour (Sept. 25-28, 1931), when *C. spectabilis* was in the early bloom, two cows stripped a considerable number of green leaves from the plants, but ate none of the stems nor flowers. They were returned to the dairy herd at the end of the 2-week tour, as originally planned. No outward symptoms of ill effects were noted immediately, nor later, when again under daily observation on the hay trials. On the basis of these earlier observations, none of the plants under trial were suspected of being toxic.

TABLE 1.—*Species of the genus Crotalaria*¹ used in palatability trials

Species	Class of roughage offered					
	Green forage		Artificially dried hay		Silage	
	1931	1932	1931	1932	1932	1933
<i>C. anagyroides</i> H. B. K.		×		×		×
<i>C. borealis</i> Guill. and Perr.						×
<i>C. granthana</i> Harvey	×	×	×	×		×
<i>C. incana</i> L.	×	×	×	×	×	
<i>C. intermedia</i> Kotschy	×	×	×	×	×	×
<i>C. lanceolata</i> E. Mey	×	×	×	×		×
<i>C. maculata</i> Klotzsch	×	×				×
<i>C. retusa</i> L.	×					
<i>C. spectabilis</i> Roth	×	×	×	×	×	
<i>C. striata</i> D. C.	×				×	
<i>C. usaramoensis</i> Baker	×	×	×	×		×

¹ × signifies use of the plant in the manner indicated; ×× signifies use of the plant twice.

Three of the cows mentioned above, and 11 heifers, were used shortly afterward in 1 or more of a series of 8 palatability trials in which artificially dried hay was used. *Crotalaria spectabilis* hay was eaten by the cows in only one of the trials mentioned above, the amount being 12 pounds. No untoward effects were noted from this amount. It may be mentioned that the cows weighed between 900 and 1,000 pounds, so that the intake per 1,000 pounds live weight was relatively small. In these palatability trials, *C. spectabilis* ranked at the bottom of a group of eight species of crotalaria hays. These observations ranged from 14 to 38 cow-days per individual animal on the grazing trials, and from 17 to 24 days per individual on the hay trials during the first year.

These palatability trials were repeated in the field and dry lot during 1932. *Crotalaria spectabilis* was rejected entirely by 5 different cows allowed access to the pasture during one or another of 5 tours. Three Jersey cows were used in the hay-palatability trials with eight species of *Crotalaria* from October 21 to November 16, 1932. These animals consistently left *C. spectabilis* in the rack as the last choice of the eight species. On November 16 three yearlings from the beef cattle herd replaced the cows in the dry lot, and at that time the latter animals were transferred to a maintenance trial on *C. intermedia* hay. Specific animals and the lengths of the tours on the several trials are outlined in detail in table 2. These summaries are compiled from the observations of cattle made daily by two or more station workers.

Four of the cows were changed from *Crotalaria intermedia* hay to corn silage and Bermuda grass (mixed) pasture on March 2, 1933. Cow no. 352 was autopsied for definite examination in search of any indications of toxicity of the forages eaten. The cows exhibited no symptoms of toxicity, nor were any gross or histological lesions evident in the organs and tissues upon autopsy, which was conducted by Drs. E. F. Thomas and C. F. Ahmann of this station. The feed of three of these cows had been limited strictly to *C. intermedia* from November 17, 1932, to March 1, 1933, inclusive—a period of 105 days, and they had had access to it previously, as noted in table 2.

TABLE 2.—Duration of feeding trials with cattle, and animals used, during the 1932 grazing season and the winter of 1932-33, the species of *Crotalaria* described in table 1 being used

Nature and duration of trial	Feedings ¹ given to animal ² indicated					
	No 151	No. 228	No 204	No 206	No 350	No 352
Palatability of green forages:						
June 18 to July 2	3 X			3 X		
July 28 to Aug. 13			3 X		4 X	
Aug. 14 to Aug. 27	4 X			3 X		
Oct. 4 to Oct. 20		4 X		3 X		
Palatability of dried hays						
Oct. 21 to Oct. 28	4 X	4 X		4 X		
Oct. 28 to Nov. 2	4 X	4 X		4 X		
Nov. 2 to Nov. 7	4 X	4 X		4 X		
Nov. 7 to Nov. 12	5 X	5 X		5 X		
Nov. 12 to Nov. 16	4 X	4 X		4 X		
Maintenance trial with <i>C. intermedia</i> :						
Nov. 17 to Dec. 16	X	X		X		
Dec. 17 to Jan. 17	X			X		
Digestion trials with <i>C. intermedia</i> .						
Jan. 18 to Feb. 11	6 X	7 X		6 X		7 X
Feb. 12 to Mar. 1	7 X	6 X		7 X		6 X

¹ X signifies feedings given on the dates mentioned

² Dates of autopsies: No 151, May 2, no 228, Mar 31, no 206, Mar 23, no 350, Apr 14, no 352, Mar 2

³ No *C. spectabilis* eaten

⁴ *C. spectabilis* rated last in relative palatability

⁵ No *C. spectabilis* hay offered

⁶ Naturally cured *C. intermedia* hay

⁷ Artificially cured *C. intermedia* hay.

No chronic symptoms had developed between the termination of the maintenance trials and the dates of autopsies of these animals. Indications of toxicity particularly sought in these cases included loss of appetite, defection, blood in the feces, and nasal hemorrhage. Gross and histological examinations upon autopsy were made particularly upon the liver, heart, kidneys, mesenteric blood vessels, and the fatty tissues. The autopsies were attended and tissues examined by four or more persons including the authors, Drs. E. F. Thomas, C. F. Ahmann, and others of the experiment station and the United States Department of Agriculture staffs. No symptoms were observed, either acute or chronic, of toxicity of the species of *Crotalaria* eaten in these trials other than *C. spectabilis*.

Three native yearling cattle—2 steers and 1 heifer—were used in continuing the test of the relative palatabilities of artificially cured *crotalaria* hays. Previous to this time, these animals had received carpet, centipede, and mixed native pasture grasses. The log of these palatability trials is given in table 3.

TABLE 3.—Relative palatability of 8 species of artificially dried *crotalaria* hays, as indicated numerically according to order of preference and rate of consumption by 3 yearling cattle in 1932

Duration of trial	Numerical palatability rating of indicated species of <i>Crotalaria</i>							
	<i>Inter-media</i>	<i>Usaramoensis</i>	<i>Incana</i>	<i>Gran-tana</i>	<i>Lance-olata.</i>	<i>Anagy-roides</i>	<i>Striata</i>	<i>Specta-bilis</i>
Nov. 16 to Nov. 23	1	2	3	4	5	6	8	7
Nov. 23 to Nov. 29	1	2	3	4	6	7	8	5
Nov. 29 to Dec. 5	1	2	3	4	5	8	7	6
Dec. 6 to Dec. 12	1	2	3	6	4	8	7	5
Dec. 12 to Dec. 19	2	1	3	7	4	8	6	5
Dec. 19 to Dec. 23	1	2	4	6	5	7	8	3
Dec. 23 to Dec. 27	2	1	4	8	5	6	7	3

The last hay-palatability trial terminated on December 27, 1932. The 3 yearling cattle used in the last 7 trials died on January 7, 18, and 20, 1933, respectively. They showed almost total loss of appetite, lack of abdominal fill, no bloat, or hoven, blood in the feces, and two had nasal hemorrhage. Autopsy disclosed hemorrhage from blood vessels in the mesentery, heart, muscle coronary and body fat, and in the mucous layer and submucosa of the trachea; a mottled appearance of kidneys and liver; and hemorrhage in the nasal sinuses of two animals.

Although *Crotalaria spectabilis* had been withdrawn from the feeding rack on December 28, 1932, the second yearling steer showed intestinal hemorrhage, as evidenced by bloody feces on January 12, 1933, which increased in severity on the 13th. The two animals surviving at this date became less animate; ate less of the crotalaria hays, and their feces were still darker on January 16. Nasal hemorrhage was apparent in the second steer on January 17. This animal was killed for autopsy on January 18, shortly prior to the approaching natural death. The yearling heifer died on January 20.

The autopsy findings, as summarized by Drs. E. F. Thomas, A. L. Shealy, and C. F. Ahmann, are as follows:

History.—Three cattle had been feeding on crotalaria hay. They showed loss of appetite for several days. Bloody feces, and a tinge of blood from the left nostril (of the second steer) were evident.

All mesenteric fat contained numerous petechial hemorrhages. Anterior portion of the frontal sinus showed ecchymotic areas; blood clots in posterior frontal sinus. A few worms were embedded in wall of esophagus (not identified). Extensive hemorrhages along the mucous membrane and submucosa of the trachea.

Endocarditis; myocarditis, and epicarditis (especially along the interventricular groove). Pericardium showed numerous petechial hemorrhages. Liver finely mottled with red. Gall bladder showed numerous petechial hemorrhages. Spleen probably enlarged slightly. Wall of abomasum showed the mucous membrane edematous. Mucous membrane of the small intestines appeared normal.

Submucosa of small intestine showed ecchymosis and petechial hemorrhage.

Large intestine.—No hemorrhage of mucous membrane, but an abundance of blood clotted in the lumen. Fecal matter was firm; some hard. Urinary bladder showed a hemorrhagic area. The brain was congested, with gelatinous material in the sulci (grooves). Lymph glands showed very little congestion. All body fat was very yellow, and showed petechial hemorrhages throughout.

Tissue from the liver, kidney, and spleen was fixed for examination.

Typical lesions of *Crotalaria spectabilis* poisoning may be seen in the left view of the heart (fig. 1), liver (fig. 2), gall bladder (fig. 3), inner surface of the trachea (fig. 4) and mesentery (fig. 5) of the yearling steer which was killed just prior to natural death. These photographs show the hemorrhages resulting from leakage of blood from the vessel walls. The lesions compare closely with those produced in chickens by Thomas, Neal, and Ahmann (10), using *C. spectabilis* seeds, and also the alkaloid extracted from the seeds, leaves, and stems of this plant.

AUTOPSY OF THIRD CASE

The third yearling (heifer) died January 20, 1933, probably about 10 hours prior to autopsy. She had been off feed for 13 days, and had passed considerable blood with the feces during the last 2 days.

The mesenteric fat showed little hemorrhage. Petechial and ecchymotic hemorrhages were present in the submucosa of the intestines. The lungs apparently were normal. There was a severe

hemorrhagic condition of the heart muscle. The liver showed a few hemorrhagic areas. The gall bladder had several ecchymotic areas, and there also were numerous ones in the spleen. The urinary bladder appeared normal. The lumen of the large intestine contained blood and blood clots. The pericardium showed numerous petechial hemorrhages. Amber fluid was present in the pericardial sac. Numerous subcutaneous petechial hemorrhages also were present.

It is to be noted particularly that none of the cows that refused to eat *Crotalaria spectabilis* in the grazing-palatability trials, or in the



FIGURE 1—Left view of heart, taken from a yearling steer affected with chronic *Crotalaria spectabilis* poisoning, showing petechial hemorrhages in the coronary fat and the outer walls of the auricles and ventricles. The hemorrhagic condition is more general in the ventricular walls adjacent to the left interventricular groove and toward the apex.

trials with artificially cured hays, showed any indications of *Crotalaria* poisoning, either in life, or upon autopsy. However, the three yearlings that changed their order of preference of these species, and consumed 98 pounds of *C. spectabilis* hay between November 16 and December 28, 1932, died of *Crotalaria* poisoning between January 7 and 20, 1933. Since they also had access to other feeds, and since there could still be a question as to *C. spectabilis* specifically, a supply of the artificially dried hay was preserved pending availability of a suitable animal for an exact trial.

SPECIFIC TEST OF *CROTALARIA SPECTABILIS*

E-53, a dun and white native steer just under 8 months of age and weighing 300 pounds, was supplied from the native herd at the experiment station. This animal had suckled its dam from birth, and had been on mowed-grass pastures during its lifetime. It had not had access to *Crotalaria spectabilis* previously, insofar as is known. The animal was healthy and extremely vigorous.

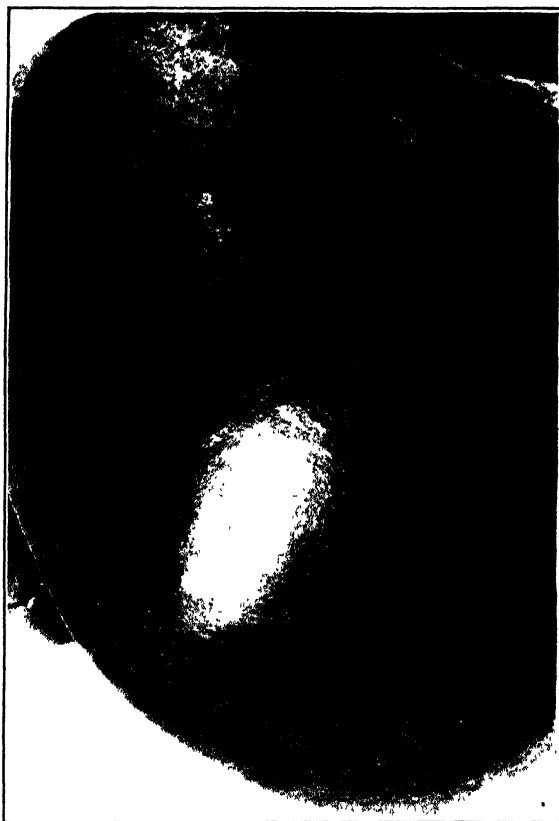


FIGURE 2.—Liver of a yearling steer affected with chronic *Crotalaria spectabilis* poisoning, showing the surface finely mottled with red as the result of hemorrhage.

Artificially dried *Crotalaria spectabilis* hay prepared in 1932, was offered to this calf, which refused to eat it. The chopped hay was mixed with other feeds, which it still refused to eat. Water was available in the dry lot at all times. A stomach tube was passed, weighed quantities of ground *C. spectabilis* hay were suspended in

water, and the animal drenched therewith. The record of these feedings of *C. spectabilis* hay was as follows:

1933	Pounds
Dec. 12.....	0.5
Dec. 13.....	4.0
Dec. 14.....	3.5
Dec. 15.....	1.5
Total.....	9.5

Blood was present in the feces the morning of December 14 and the animal died about 4:30 p. m. the next day.

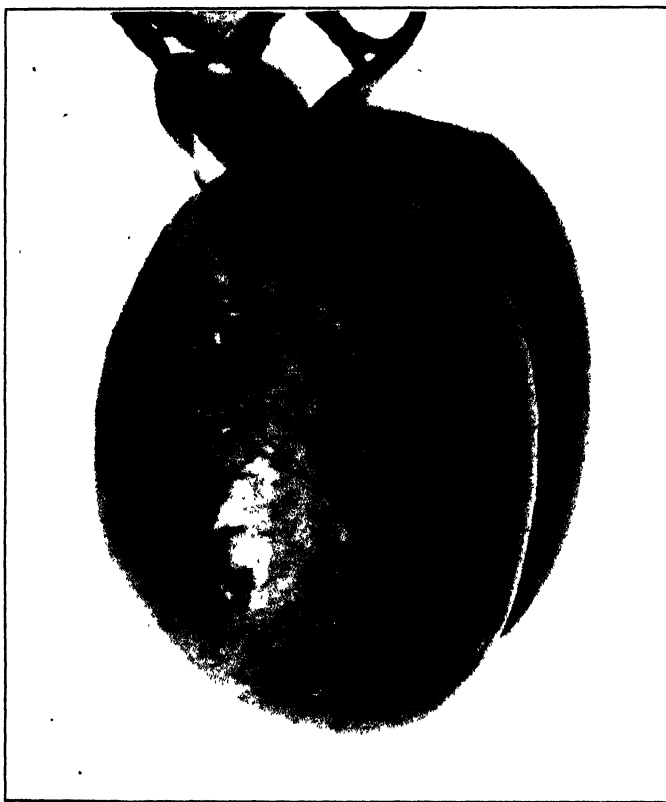


FIGURE 3.—Gall bladder from a yearling steer affected with chronic *Crotalaria spectabilis* poisoning, showing numerous petechial hemorrhages. The bile appeared normal in color and consistency.

E-53 was photographed a few minutes prior to death (fig. 6). Death occurred quietly, the animal lying on its side, the only struggle being a slight paddling of the feet. The autopsy was made on the warm body before the onset of rigor mortis.

The blood had not clotted, nor did it clot readily upon the severing of the jugular vein. The subcutaneous blood vessels, those in the adipose tissue and along the small intestines, were enlarged. The thymus and spleen appeared normal. A slight amount of straw-

colored fluid had accumulated in the peritoneal cavity. Urine in the partly filled bladder was of a normal pale-amber yellow color.

Petechial hemorrhages were present in the mesentery, on the caecum, and in the gall-bladder wall. Mesenteric lymph glands were enlarged. The lumen of the lower small intestine, all of the large intestines, and rectum contained the bloody material mentioned previously as appearing in the feces. No hemorrhages were apparent on the stomach compartments nor in the lungs. The kidneys were

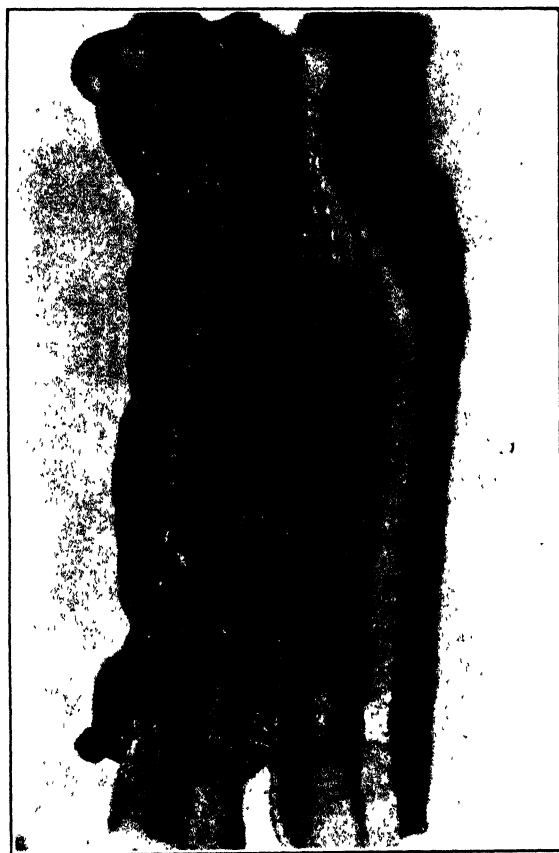


FIGURE 4 Inner surface of the trachea of a yearling steer affected with chronic *Crotalaria spectabilis* poisoning, showing extensive hemorrhages along the mucous membrane and submucosa of the trachea. The trachea was opened along the ventral surface.

darker, with a slight turkey-egg mottling. The liver also was a dark red-blue color, and quite friable. The gall bladder wall was thickened and spongy in texture. The bile appeared normal in color and consistency.

The heart showed petechial hemorrhages in the coronary fat, on the auricles and along the interventricular groove. Parts of the inner wall of the heart, the papillary muscles and some of the chordae tendineae showed marked hemorrhages, as seen in figure 7.

The case of E-53 is considered acute, whereas those of the three animals dying previously were chronic.



FIGURE 5—Mesentery and intestines of a yearling steer affected with chronic *Crotalaria spectabilis* poisoning, showing numerous petechial hemorrhages in the mesenteric fat, on the outer surface of the small intestines and the apex of the caecum. The submucosa of the small intestines showed ecchymoses and petechial hemorrhages. There was an accumulation of clotted blood within the lumen of the large intestines



FIGURE 6.—An 8-month-old steer photographed less than half an hour before dying from acute *Crotalaria spectabilis* poisoning. Loss of appetite and lack of animation were evident in the acute and chronic cases alike. Dried blood from a nasal hemorrhage is visible below the right nostril. The hair is harsh and unkempt.

TRIALS WITH SILAGE

In 1932 four species of *Crotalaria*, including *C. spectabilis*, were ensiled in 1-ton laboratory silos (7) for use in comparing the relative palatability of these species. *C. intermedia* was first choice of the

cattle, and was eaten liberally. The *C. spectabilis* ranked third in order of preference by the cattle. The latter silage had a dark color, and animals eating it voided dark-colored feces. These silages were offered to 30 cattle over a 16-day period. No irregularities were observed with these cattle other than the temporary discoloration of the feces.



FIGURE 7.—Inner walls of the heart of a 300-pound steer that died of acute *Crotalaria spectabilis* poisoning. There are extensive hemorrhagic areas on the inner lining of the ventricles, the hemorrhages extending deeply into the heart muscle. The papillary muscles and a few of the chordae tendineae also show hemorrhages.

Two palatability studies conducted in 1933 dealt with six species of *Crotalaria* as silage. These were of 11 days' duration each, and involved 51 cattle. As noted in table 1, no *C. spectabilis* was offered at this time, nor were indications of toxicity observed in the cattle during, or after, eating these species of *Crotalaria* as silage.

Twenty-five tons of *Crotalaria intermedia* were ensiled in a commercial silo, and fed to dairy cows in a 90 days' double-reversal feeding trial, and to four steers over periods of 59 and 31½ days in a study of coefficients of digestibility. No indications of either acute or chronic toxic influences were observed in any of the cattle used in investigations on the *C. intermedia* silage.

SUMMARY

Grazing and feeding trials indicate that at least 8 out of 10 introduced species of *Crotalaria* are probably not toxic to cattle. *C. retusa* was not grazed. *C. spectabilis* is definitely toxic to cattle.

One acute and three chronic cases of *Crotalaria spectabilis* poisoning in cattle are discussed, with symptoms and lesions as noted. *C. spectabilis* Roth is added to the list of species of this genus definitely proved toxic to domestic animals.

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CLASSIFICATION OF CHICK-EMBRYO POSITIONS AT DIFFERENT AGES AND MALPOSITION AS A CAUSE OF MORTALITY¹

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INTRODUCTION

Growth of the chicken embryo is accompanied by gradual changes in position in accordance with the necessity of breaking through the shell and shell membranes at hatching time. Failure to secure a correct position with the beak near the air cell, the egg tooth directed toward the shell, the pipping muscle free to function, and the feet in a position for body rotation, may either hinder or prevent escape. As a consequence the ineffective position would appear to constitute a mechanical lethal factor, and malposition would be considered a cause of death.

A direct causal relationship between malposition and embryonic mortality has been considered by Sanctuary (6),² Hutt (3), Hutt and Cavers (4), and others. Hutt and Pilkey (5) have observed malpositions in more than 50 percent of the "dead in shell" embryos. They state that malposition is "largely responsible for the peak of mortality occurring during the last 3 days of incubation."

Nevertheless, the causal relationship that is supposed to exist between position and death has been defined only in part. Byerly and Olsen (1, 2) have shown that both gravity and air hunger effect changes in some of the positions of the embryo. Hutt and Pilkey (5) consider position of the egg during incubation and orientation of the primitive streak important factors in effecting malposition. But these malconditions are not commonly met when proper methods of incubation are followed. Position I as indicated by Sanctuary (6), with the head between the legs, may be produced artificially by inverted incubation through the effects of gravity and of air hunger. That does not furnish an explanation for the cause of its occurrence under nonexperimental conditions with proper methods of incubation applied.

Furthermore, all six malpositions so far reported (table 1) have been recorded from the dead-in-shell. A classification of the various positions taken by normal embryos, that is, the positions that do not interfere with hatching, has never been recorded, as far as the writer is aware. Yet, before a causal relationship can be considered as existing between embryo position and embryo mortality, i. e., before a position can be termed malposition in the sense that it hinders or precludes hatching, a knowledge of the relative occurrence of positions taken by normal chicks is essential.

¹ Received for publication Feb. 14, 1935; issued July 1935.

² Reference is made by number (*italic*) to Literature Cited, p. 931.

TABLE 1.—*Malpositions of chick embryos*¹

Position no.—	Described by—	Description
I.....	Sanctuary, 1925.....	Head between the thighs.
II.....	{ do.....	} Head in small end.
III.....	{ Reaumur, 1751.....	
IV.....	{ Sanctuary, 1925.....	} Head to left instead of under right wing
V.....	{ Hutt, 1929.....	
VI.....	Smith, 1930.....	Head normal, but embryo rotated so that the beak is buried away from the air cell.
VII.....	Hutt and Cavers, 1931.....	Feet over head.
		Beak over right wing or over and under wing

¹ Hutt and Pilkey (5, p. 4).

METHODS AND PROCEDURE

In connection with a study of the causes of embryonic death instigated by the writer at the Maine Agricultural Experiment Station in 1927, a systematic mortality code has been developed to record teratas and defects of embryos or of embryonic tissues which might in any way give a clue to the causes of death. At the same time, as supplementary information on the cause of death, data have been accumulated on the positions of the embryos.

Early in the work it became evident that malposition is not in itself always a cause of death, (1) because position was found to vary with age (the position of a 15-day-old embryo retained by an embryo 21 days old indicated, among other possibilities, a retarded development), and (2) because there appeared a large number (upward to 12 percent) of obviously defective embryos. These defective embryos were in characteristic abnormal positions, but their failure to hatch was a result of abnormal development and not of malposition. In fact, defective embryos occurring most frequently (termed A3) were characterized by nanism and torticollis with the head twisted sideways or even backwards. The failure to emerge was not the result of an awkward position, since the embryos usually had failed to assimilate all of the albumen, were lethargic, and lacked directive movement. Granted that their positions were not advantageous to emergence, it would have been impossible in any case for such individuals to hatch.

If the position of the embryo is to be used as a means of determining the causes of failure to hatch, it is necessary to determine first the cause of each position type, i. e., whether or not it actually prevents the act of pipping the shell or is an earmark of a more deeply-seated physiological defect.

As an approach to this phase of the subject a classification was made of the orderly changes in position of embryos with age. The purpose was threefold: (1) To obtain a position type for each age, (2) to obtain information on the variability of positions so that malposition may be more clearly defined, and (3) to observe the process and possible causes for the different position types. This paper indicates the order of changes in position of embryo from the fifteenth day of incubation to the twenty-first, and presents a systematic chart of those changes together with the variations in position that an embryo may take whether normal or abnormal. The chart links all six positions previously recorded by other workers with the new positions found by the writer and includes a prediction of positions to be found by following through the mirror image of the positions so far discovered.

SYSTEMATIC CLASSIFICATION OF POSITION TYPES

A systematic classification of embryo position which covers all possibilities is sometimes difficult to accomplish, since unsuspected position types appear in new sources of material. Consequently a flexible classification was made that could be expanded if necessary.

The code is based upon a division of positions into independent events, the occurrence of any one of which does not occlude the occurrence of any other, and the subdivision of each independent event into mutually exclusive events, only one of which may occur in any embryo.

The four independent groupings are as follows:

- A. Position of the air cell in respect to the egg.
- B. Orientation of the embryo as a whole within the egg.
- C. Position of the head, beak, and neck in relation to the body.
- D. Position of the beak in relation to the air cell.

The subdivisions of each independent group into mutually exclusive events or positions are listed in table 2.

From the foregoing classification it is possible to describe with exactitude the position of any embryo from 15 to 21 days of age. Thus the normally orientated embryo is described as 1-a-6-a:

- 1. Air cell in base of egg (large end).
- a. Embryo with head in large end of egg.
- 6. Head turned to right side, with beak under right wing.
- a. Beak near air cell.

There are a few unclassified conditions that may interfere with hatching. These are listed under abnormal feet positions and include the following:

- 1. Agrippa, feet first.
- 2. Legs crossed.
- 3. Feet tied by membranes or by the yolk stalk.
- 4. Membranes tied about the body or neck.
- 5. Hernia due to kicking.
- 6. Broken yolk sac due to kicking.

The foregoing accidental defects have been placed under a separate classification.

TABLE 2.—Code for the classification of chick-embryo positions, including a code for the orientation of the embryo in respect to the egg axis and the air cell, based on four independent events

A, air cell	B, body position	C, position of head and beak, and direction of neck twist			D, proximity of beak to air cell
		Head	Beak	Primary twist in neck	
1, in base.	a, head in base.	0, between legs, feet over head.	Straight down	None	a, beak near air cell
2, in tip.	b, head in tip.	1, to center between legs	do	None	b, beak away from air cell (1 inch or more).
3, at side.	c, head and feet in tip.	2, to center between legs	Tip to right		
	d, head and feet in base	3, to center between legs	Tip to left		
		4, across breast	Points to right	Sinistral	
		5, across breast	Points to left	Dextral	
		6, to right side	To right, tip under right wing	Sinistral	
		7, to left side	To left, tip under left wing	Dextral	
		8, to right side	To right, tip under and over right wing	Sinistral	
		9, to left side	To left, tip under and over left wing	Dextral	
		10, to right side	To right, tip over right wing	Sinistral	
		11, to left side	To left, tip over left wing	Dextral	
		12, on right side	To front and right	Do	
		13, on left side	To front and left	Do	
		14, on right side	To back or above	Sinistral	
		15, on left side	To back and above	Right torticollis	
		16, to front	To front and above	Left torticollis	
		17, to back (above and over)	To back (above and over)	S-curve forward and upward Retrocollis	

NORMAL CHANGE IN POSITION OF THE EMBRYO WITH AGE

During the season of 1931 observations were made on the normal change in position taken by the embryo as it advances in age from a small floating primitive streak to a hatching chick tightly packed in the shell. Positions were classified according to the scheme presented in table 2. For the effect of age, consideration was given only to head, wing, and neck positions with all other factors constant. The position frequencies for each age, from 15 to 21 days of age, are shown in table 3.

TABLE 3.—Changes in head position of chick embryos with incubation age and frequency of the various head positions

Incubation age (days)	Head between legs, 0-1-2-3 ¹	Head across breast, 4-5 ¹	Wry neck, 12-17 ¹	Normal, 6-8-10 ¹
15	2 17	3		
16	2 50	15	4	
17	83	2 106	13	
18	33	2 58	9	4
19	4	2	2	2 12
20	4			2 14

¹ Code numbers refer to the embryo positions described in column C in table 2
— modal class.

The results show that head positions 1, 2, and 3 predominate in embryos from 15 to 16 days of age, that head positions 4 and 5 pre-

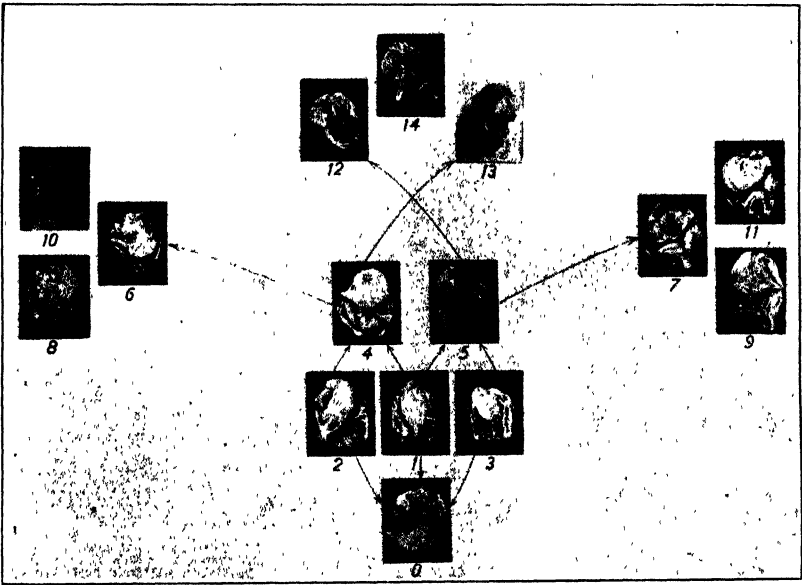


FIGURE 1 Codification of head positions of 21-day-old chick embryos. 0, Extreme form of 1, 2, 3, 1, 2, 3, similar to 15- and 16-day-old; 4, 5, similar to 17- and 18-day-old; 6, typical hatching position, 8, 10, variations of position 6, 7, 9, 11, reverse of positions 6, 8, 10; 12, 13, 14, defective.

dominate in embryos from 17 to 18 days of age, and that head positions 6, 8, and 10 are the most common for embryos 19 days old and over.

In other words, the head position is at first (at 15 days of age) straight down between the thighs nearly to the tail. The tipping of the beak to the right or to the left apparently indicates the direction the beak will take even before the head is raised to the side of the body. At 17 days the head is drawn up over the breast where it turns either to the right or to the left according to its normal tendency. At 18 days the head lies across the breast.

TABLE 4.—*Frequency distribution of embryo positions*

Code ¹	Hatch- able ² alive, 1931	Pipped, dead, 1931-32	21 days old, dead, 1931-32	18 to 20 days old, dead, 1931	20 + 21 + pipped dead, 1932		Abnor- mal 21 days old, 1931-32
					Male	Female	
A, air cell							
1.	218	339	942	168	375	423	157
2.		1	2		1		
3.		1	9	1	4	6	1
9.		3		1			
B, body position							
a.	212	335	891	151	342	393	141
b.	6	7	44	15	29	33	8
c.		1	14	3	9	2	4
d.			4	1		1	4
9.		1					
C, head position							
0.			1		1		
1.			18	7	4	10	4
2.		2	60	12	14	17	7
3.			72	14	18	13	5
4.		9	79	16	34	47	42
5.		1	40	7	10	15	4
6.	125	217	218	53	143	144	11
7.			104	12	31	34	4
8.	27	63	27	1	23	29	13
9.			2			4	
10.	66	51	192	32	64	75	5
11.			4			1	3
12.			111	13	25	37	41
13.		1	22	3	17	4	16
14.			1				1
15.			1				
16.							
17.			1		1		1
D, beak to air cell							
a.	212	428	518	96	230	263	44
b.	6	30	444	73	150	166	110

¹ Coded according to description in table 2 under columns A, B, C, D.

² From examination of chicks as they hatched. A few might have died as "pipped."

Head positions 4 and 5 of the 18-day-old embryos are in critical stages, since they are the axes about which and on which the final position of the hatching chick depends. An embryo at 18 days of age with its head across the breast (4) with tip of beak pointed to the right has an alternative of five different positions on the nineteenth day, i. e., either to slip backwards to the left and form the position 13 or 15 (the wry-neck class) or to slip to the right and form the so-termed "normal position" (6), with beak protruding under the right wing, or one of the variants (8) or (10).

Should the beak be pointed toward the left, the opposite directions would be taken with five different positions among the left possibilities (mirror images of the right sides). In some wry-neck embryos (torticollis) the head continues to move backward along either side (positions 14 and 15). Another class of wry-neck embryos (retrocollis) appears with the head thrown straight back (positions 16 and 17). Torticollis also appears in embryos 14 days old or younger, apparently without going through this serial change in position.

Table 2 is made to conform with the logical changes in head position taken by the embryo as it develops from the fifteenth day. In figure 1 are shown all of the head positions found in 21-day-old embryos. Attention must be called to the fact that among these mature embryos will be found head positions 1, 2, and 3, which are typical for the 15- and 16-day-old embryos. As 21-day-old embryos they are in retarded positions. In the same way head positions 4 and 5 indicate a partial retardation since they are typical for embryos of 17 and 18 days of age. Head positions 12, 13, and 14, on the other hand, are the positions taken by defective embryos. Head positions 15, 16, and 17 have been found in defective embryos which died before the twentieth day of incubation. In table 4 the frequency distribution of embryo position is listed for each independent event, and again for each mutually exclusive head position as taken by various types of embryos: By normal hatchable chicks, by those that pipped and died in the shell, by those that died at from 18 to 21 days of age, in certain cases by males contrasted with females, and by those abnormal at 21 days of age.

POSITION OF ABNORMAL EMBRYOS

Abnormal embryos, like the A3 previously mentioned, together with extreme examples of chondrodystrophic embryos, are listed as to position under the term abnormal in table 4. As a group they do not differ significantly from other types of embryos in regard to air cell or body position. But in head position they are most frequently classified as partially retarded (head position 4) or as wry-neck (head positions 12 and 13). They also have a general disregard for the air cell since in over 70 percent of the cases the beak is not near the air cell. This figure is in distinct contrast to the percentage of hatchable chicks and pipped chicks which failed to find the air cell, i. e., 3 and 7 percent respectively.

DETERMINATION OF THE CAUSE OF FAILURE TO HATCH FROM THE POSITION OF THE EMBRYO

Contrary to the statements cited that "malposition is one of the primary causes of embryo mortality" the results of this analysis show that a large portion of the embryos that fail to hatch are in positions which can be considered as the secondary effect of some more fundamental defect. The correlation assumed to exist between these positions and mortality is therefore, at times, spurious. As an example of the fallacy *post hoc ergo propter hoc*, it may be likened to the inference that since rabbits poisoned in their burrows by strychnine are found in strained and peculiar positions, they must have failed to escape from their warren and from death because of these eclamptic positions. In a similar manner the fundamental causes of the defective condition in an embryo, whether they be from the effects of inbreeding, of faulty nutrition, or of careless incubation methods, will interfere with the normal sequence of body shiftings and as a consequence, will leave the embryo in a retarded or strained position when death occurs.

Malposition I of Sanctuary (6), which is coded here as head positions 1, 2, and 3, together with the extreme form, malposition V of Smith (7), (coded here as head position O) may now be used to indicate

three possible causes of death: (1) Air hunger when the air cell is misplaced, (2) gravity when the egg is incubated upside down, and (3) factors delaying development previous to the fifteenth day of incubation. Likewise head positions 4 and 5 appear to be due to the same causes to a lesser degree.

The third cause of death, i. e., that which manifests itself by delayed development, has not heretofore been considered a factor in causing malposition. It is obvious from table 3 and table 4 that delayed development in whatever form it may occur is a phenomenon associated with embryonic mortality.

Attempts to bring about a normal position in such embryos are wasted. A slight degree of debility will cause an embryo to vacillate in taking a normal position regardless of the artificial attempts to turn the egg and force it into a more appropriate orientation. Attempts to force such malposed embryos by egg turning will result only in another type of malposition.

Thus when Hutt and Pilkey (5) found that tilting the eggs during incubation produced an increase of 2.10 percent in positions I, III, and VI, they found that horizontal incubation resulted in an increase of 2.73 percent in positions II and IV. Their attempts to combine the advantages of both methods of turning should prove futile if we look upon these malpositions as a form of general debility. In such a case the correcting factor might be found in feeding to increase vigor of the embryo rather than in methods of turning the eggs during incubation. These slightly retarded embryos, together with the obviously defective embryos, constitute a large portion of the dead in shell. Their positions may suggest some of the causes for death, but the positions do not as such form barriers for their emergence.

Malposition VI of Hutt and Cavers (4), which is coded here as head positions 8 and 10, occurs in 21-day-old embryos failing to hatch in approximately 42 percent of the cases. But the same positions are found to occur among hatchable chicks with the same frequency, i. e., 42 percent (table 4). Consequently these 2 head positions appear to be variations of the more typical normal head position 6 and should therefore not be termed malpositions.

SUMMARY AND CONCLUSIONS

A systematic classification of the positions taken by normal hatchable chick embryos and by the dead-in-shell shows that position as such is not always, and may only occasionally be, the real cause for failure to hatch. Position VI of Hutt and Cavers (4) has been found to occur in hatchable chicks with a frequency equal to that found in dead-in-shell chicks; it cannot therefore be termed a malposition. Position III of Sanctuary (6) and position V of Smith (7) have been shown to be due to a delayed development taking effect prior to the fifteenth day of incubation. Defective embryos in new positions have been associated with nutrition of the dam. Thus the correlation between position and embryo mortality is in part spurious. Postdiction of causes for failure to hatch from position of the embryo become apparent only after the conditions are resolved into more fundamental causes, i. e., effects of breeding, of feeding, and of incubation methods, many of which bring about a retardation in the normal shift in position as a secondary after-effect of the true lethal factor.

A position code is presented for the classification of embryos. It is based upon a combination of independent and mutually exclusive events so that the position of any embryo may be easily described by means of a four-letter code.

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PHYSIOLOGICAL STUDIES OF SEVERAL PATHOGENIC BACTERIA THAT INDUCE CELL STIMULATION IN PLANTS¹

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INTRODUCTION

The nature of the stimuli responsible for atypical and pathological growth of living cells is of extensive biological interest. A group of closely related diseases caused by bacteria which induce such growths in higher plants seems to offer favorable material for studies in this field. Of these diseases, crown gall has received the most attention. It was first studied intensively by E. F. Smith and his associates, who considered it to be similar in many respects to certain types of sarcomata and who referred to it as plant cancer. Riker and Berge (15)³ have made a critical review of the literature on crown gall and have pointed out certain advantages offered by plant material for fundamental studies on the nature and cause of such growths. They have suggested methods of testing the hypotheses put forward by workers investigating the cause and nature of stimuli concerned with atypical and pathological growths. The present paper, an abstract of which has already been published (11), and which reports the results of a unit in a larger Wisconsin project, is concerned with various aspects of the comparative physiology of a group of cell-stimulating plant pathogens.

The plant pathogens examined in this study were, for the most part, well known. They were *Phytophthora tumefaciens* (Smith and Town.) Bergey et al., which causes crown gall of plants; *P. beticola* (Smith, Brown, and Town.) Bergey et al., the causal agent of bacterial pocket rot of sugar beets which is designated as beet gall in this paper; *P. savastanoi* (E. F. Smith) Bergey et al., var. *nerii* C. O. Smith, which causes oleander tubercle or gall; *P. savastanoi* (E. F. Smith) Bergey et al., the causal agent of olive knot; and raspberry cane gall, an unnamed bacterium producing overgrowths on the canes of black raspberry plants.

The diseases caused by these pathogens are characterized by large overgrowths composed of hypertrophic and hyperplastic tissues often containing disorganized vascular elements and sometimes conspicuous pockets of necrotic tissue. Elcock (6) elaborated on the intercellular

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² The writer wishes to express his indebtedness to Dr. A. J. Riker, under whose direction this work was done, for helpful suggestions and constructive criticisms during the progress of the investigation and the preparation of the manuscript. Grateful acknowledgments are also made to Drs. G. W. Keitt and I. L. Baldwin for helpful advice, and to Eugene H. Herrling for preparation of the illustrations.

³ Reference is made by number (italic) to Literature Cited, p. 951.

position of the bacteria in galls of sugar beets earlier mentioned by E. F. Smith et al. (20). Similarly, C. O. Smith (18), working on oleander tubercle, found the same bacterial position in connection with this disease. E. F. Smith (19) had studied the olive knot disease and found the bacteria in the intercellular spaces of the host, together with abnormal tissue development and bacterial cavities. Recent unpublished work by W. M. Banfield on the raspberry cane gall disease showed this malady to be closely related to the others with respect to cellular stimulation of the host and the position of the bacteria in the intercellular spaces. Since the bacteria causing crown gall (12, 13, 16) were found primarily in the intercellular spaces of the host, it appeared that this disease was correspondingly similar to the others and that one of the chief differences, the formation of necrotic pockets, was mostly one of degree.

After a consideration of these morphological similarities of the host-parasite relations, and of the pathogenicity of the various cultures, a comparative study of certain physiological characteristics of the causal organisms was undertaken.

IDENTIFICATION OF CULTURES EMPLOYED

The cultures of bacteria employed in this study were obtained through the courtesy of several investigators as follows: (1) The crown gall organism, *Phytoplasma tumefaciens*, came from A. J. Riker. Its earlier history (14) was given under the designation A-1. (2) The cane gall organism was secured from Banfield (3) who isolated it from a gall on a black raspberry stem grown in northern Indiana. So far as the writer is aware, this organism has not been named. (3) The beet gall organism, *P. beticola*, was obtained from A. J. Quirk. This culture was a "rough type" as described by Brown (4). (4) The oleander organism, *P. savastanoi* var. *nerii*, was secured from diseased material collected in California by C. O. Smith. (5) The olive knot organism, *P. savastanoi*, was obtained from diseased material collected in California, by the H. J. Heinz Corporation.

Isolations from the diseased materials were made in the usual way, and the pathogenicity of all of the cultures established before further purification was undertaken. The details of this work are omitted because of their volume.

Single-cell cultures were made according to the methods of Wright et al. (23), with slight modifications suggested by A. A. Hendrickson. Yeast-extract-manitol-mineral-salts liquid medium (described later) was used. The parent cultures were incubated 18 to 20 hours at 24° C. before isolations were attempted. Microdrops from this culture were made beside large drops of sterile media on sterile cover slips until an individual cell appeared. If more than one cell was placed in the microdrop, the cover slip was discarded. The microdrop was incubated for 24 to 48 hours before it was mixed with the adjacent large drop.

The pathogenicity of the single-cell cultures was determined through a series of inoculations on suitable hosts. The details of this work also are omitted because of their volume.

CROSS INOCULATIONS

Cross inoculations were made with these cultures to determine their pathogenicity in the plants used. The ability of each organism

to parasitize the hosts listed in table 1 was tested (1) with cultures from poured-plate isolations, (2) with progenies of single-cell cultures, and (3) with bits of gall tissue from each host. Since the results with these different methods were similar, they are considered together. The inoculations were made and repeated during the winters of 3 consecutive years on plants grown in the greenhouse at approximately 24° C. Only vigorously growing organisms and plant materials were used. The incubation period ranged from a minimum of 5 days for the beet gall organism on garden beets to a maximum of 20 days for the olive knot organism on olives. Failure to produce infection on a suitable host under the above-described conditions was rarely encountered even with organisms kept over 3 years in culture.

TABLE 1.—*Results of inoculations with various gall-forming bacteria on several plants*

Organisms with which inoculations were made	Pathogenicity ¹ on —				
	Bonny Best tomato	Cumberland black raspberry	Detroit dark red beet	Pink and white oleander	Manzanillo olive
Crown gall	+	+	+	+	+
Cane gall	0	+	0	0	0
Beet gall	0	0	+	0	0
Oleander gall	0	0	0	+	+
Olive knot	0	0	0	20	+

¹ Positive results indicated by +, negative results by 0. During the course of this work over 100 inoculations with the organism on its proper host and over 20 inoculations of each organism on each host were made.

² One of the twenty inoculations suggested positive results.

The results of these inoculation studies are summarized in table 1. No differences worthy of note were observed either in different trials or with the various sources of inoculum. Portions of this work confirm that of E. F. Smith (19) and C. O. Smith (18). The details are omitted because of their volume.

PRELIMINARY PHYSIOLOGICAL STUDIES

A further means of identification was obtained by growing the various pathogens on the substrata mentioned in the literature, which provided characteristic reactions.

The results of these studies are summarized in table 2. As far as this work was carried, the results agreed closely with those of Brown (4), Riker (14), C. O. Smith (18), and E. F. Smith (19). It was also suggested that the undescribed cane gall organisms might be distinct from all the others because (1) it was very slow growing in all the media, and (2) it produced a different type of growth in litmus milk, characterized by a slight serum zone, pink color, acid reaction, and curd after an incubation period of 3 weeks at 24° C. Further differences appeared during the investigations on carbon and nitrogen utilization mentioned later. None of these media showed clear differentiation between the bacteria causing oleander gall and olive knot. C. O. Smith (18) concluded that these two organisms were almost identical as far as his cultural studies were carried.

TABLE 2. Summary of results obtained with cultures of various gall-forming bacteria on different media

Test and media	Results with organism causing—				
	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot
Growth on (in)—					
Nutrient glucose agar	++	+	+++	+++	+++
Nutrient glucose broth	++	+	+++	+++	+++
Nutrient agar	++	+	+++	+++	+++
Yeast-extract-glucose agar	++	++	+++	+++	+++
Yeast-extract-glucose liquid medium	+++	++	+++	+++	+++
Potato-glucose agar	+++	++	+++	+++	+++
Litmus milk	++	++	+++	+++	+++
Serum zone	Present	Present	Present	None	None
Color	{ Gray to brown } Alkaline	Pink Acid	{ White to brown } Acid	Blue Alkaline	Blue Alkaline
Reaction					
Liquefaction of gelatin ¹	0	0	+	0	0
Hydrolysis of starch ²	0	0	+	0	0
Digestion of casein ²	0	0	+	0	0

¹ Amount of growth indicated as follows: +, slight; ++, moderate; +++, abundant

² Results indicated as follows: 0, negative; +, positive

COMPARATIVE PHYSIOLOGICAL STUDIES

METHODS AND APPARATUS

The bacteriological work reported in this paper was performed in a special room with the temperature controlled at approximately 24° C. The air of the room was supplied through oiled-paper air filters which kept dust and contamination at a minimum. The methods of the Society of American Bacteriologists (21) were employed unless otherwise noted. The bacterial suspensions used in seeding the media were prepared according to a standardized technic as follows: The density of a water suspension from a 2-day-old potato-glucose agar culture was adjusted to approximately 1.0 of the McFarland (8) scale. The cultures were seeded in duplicate with one drop of suspension and allowed to incubate 21 days at the temperature of the room. Experiments were repeated at least three times unless otherwise noted. Special technics are described under appropriate headings.

The selection of a synthetic basic medium which contained suitable sources of carbon and nitrogen readily available to all of the cultures and which would support growth without releasing objectionable end products was desired. Yeast-extract-glucose-mineral-salts medium, while not possessing all the above requirements, proved satisfactory. Its composition was as follows: 1-percent yeast extract, 100 cc; glucose, 5 g; magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.2 g; calcium chloride, (CaCl_2), 0.1 g; sodium chloride (NaCl), 0.2 g; dipotassium phosphate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$), 0.2 g; and water, 900 cc. The reaction was adjusted to approximately pH 7.0. The yeast extract was made according to the method of Allyn and Baldwin (2). Tests for the presence of reducing sugars in the yeast extract both before and after hydrolysis were negative. However, a trace of growth was made by the bacteria, as may be seen later when the glucose was omitted. Attempts to prevent this growth by fermentation of the yeast infusion with *Bacillus coli communis* were unsuccessful.

Hydrogen-ion concentrations were measured potentiometrically by means of a glass electrode together with a vacuum-tube circuit slightly modified from that described by Partridge (10). A diagrammatic representation of the equipment together with the electrode assembly is given in figure 1.

The glass electrodes or half cells were made essentially by the procedure of MacInnes and Dole (9).

Calibration was effected by determining the electrode potentials of various standard buffers covering the pH range of the experiments studied. The error in the measurements, between pH 1.5 and 11.0, was found to be less than ± 0.02 pH unit for any single

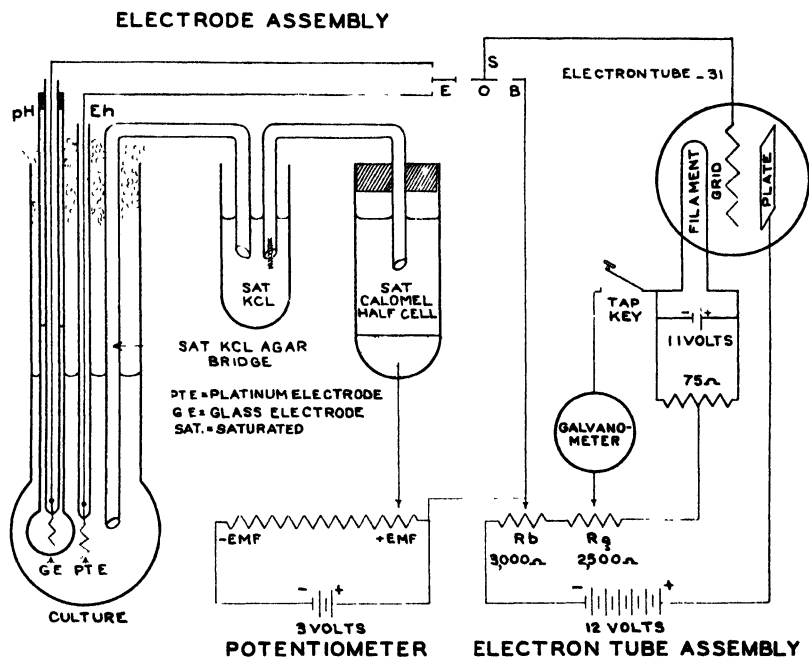


FIGURE 1. Diagram of vacuum-tube galvanometer and electrode assembly for measuring oxidation-reduction intensities and hydrogen-ion concentrations with the platinum and glass electrodes, respectively. With switch *S* in the *O* position, the variable resistance *R_g* is adjusted with the aid of the tap key until the galvanometer reads zero. Switch *S* is then placed in the *B* position and the variable resistance *R_b* is adjusted until the galvanometer again reaches zero. Both these positions of switch *S* are checked again. When they are balanced at zero, switch *S* is then moved to the *E* position. The electrode potentials of the platinum or glass are read from the potentiometer in the usual manner and the values interpolated to *E_h* or pH, respectively.

experiment. These limits of error were determined by calibration before and after each experiment. All measurements were recorded to the nearest 0.1 pH unit.

Oxidation-reduction intensities were measured potentiometrically by substituting two bright platinum electrodes for the glass half cell; no other modifications of the apparatus were necessary. The operation of similar apparatus has been described by Allyn and Baldwin (2) and by others.

The yeast-extract-glucose liquid medium as employed in these trials showed little change in oxidation-reduction intensity whether it

was open to, or sealed from, the air. Figure 2 (control) shows the slight changes that occurred during a period of 21 days. Under other conditions Allyn and Baldwin (2) show significant time-potential changes in sterile media of various other types. The differences noted might perhaps be accounted for by an incomplete seal over the closed

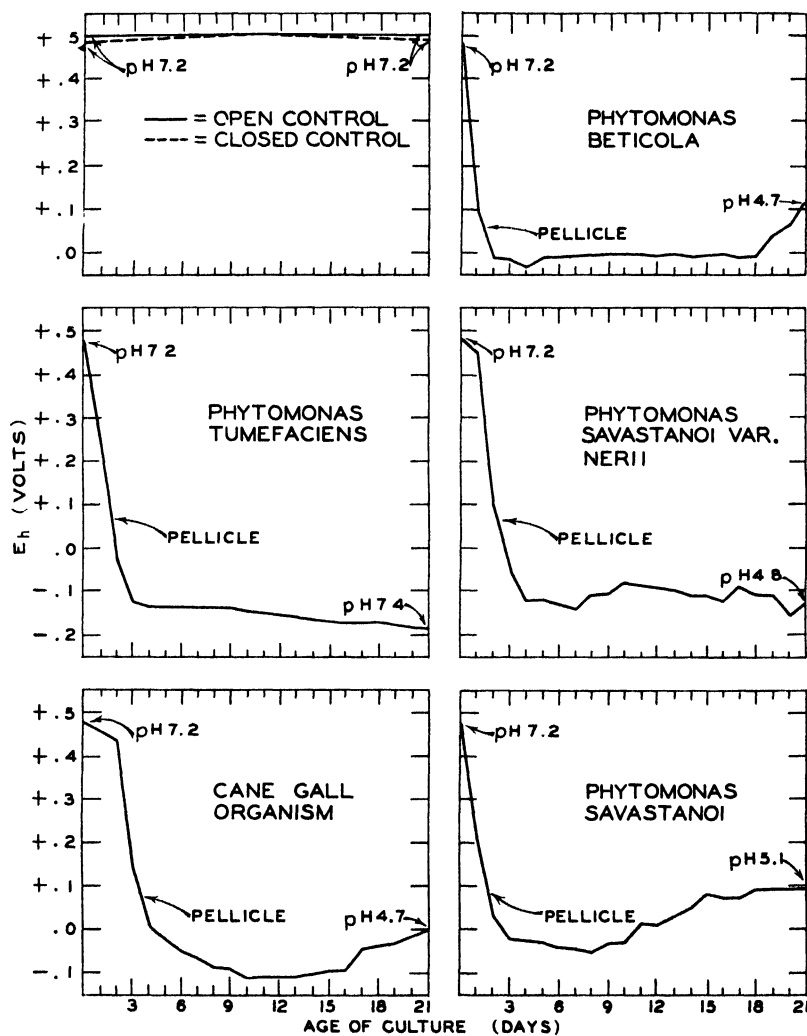


FIGURE 2 —Action of five gall-forming plant pathogens in relation to time on oxidation-reduction intensity, measured in volts (E_h), change in pH, and pellicle formation in yeast-extract-glucose-mineral-salt liquid medium at 24° C

tubes (fig. 2), or by the use of yeast extract by the writer which had either stood a long time or had been heated enough to destroy its capacity for further reduction.

Changes in hydrogen-ion concentration of the sterile basic medium were reflected in the platinum electrode potentials. Figure 3 shows

the observed E_h of their sterile basic medium at stated pH values. The readings through the physiological range of pH fall approximately on a straight line. For each unit change of hydrogen-ion concentration between pH 2.0 and 11.0 the average observed change of the platinum-electrode potential was approximately 48 millivolts. Clark and Cohen (5) in a study of the theoretical effects of pH on E_h found for each unit change of pH a possible shift of from 0 to more than 60 millivolts in the physiological range. Obviously oxidation-reduction systems are comparable only when hydrogen-ion concentrations are considered.

Autoclaving the platinum electrodes in distilled water was observed to increase their potentials in the basic medium over clean but unsterilized electrodes. A change of 50 to 60 millivolts toward the more oxidized region was noted. Similar changes were noted by Allyn and Baldwin (2) between electrodes autoclaved in steam and in water.

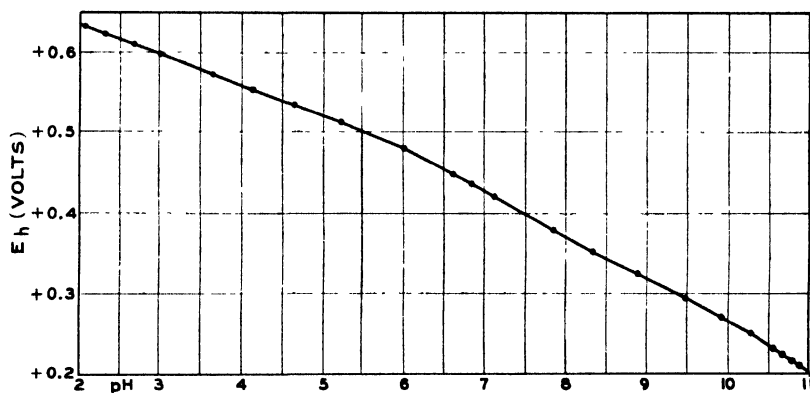


FIGURE 3 The relation between hydrogen-ion concentration and oxidation-reduction intensity in yeast-extract-mineral-salts-glucose liquid medium at 24° C. This curve was obtained by titrating the medium with standard hydrochloric acid in the acid range and with sodium hydroxide in the alkaline range. The E_h and pH were read potentiometrically with the platinum and glass electrodes described in the text.

The use of more than one electrode was obviously necessary since an erratic behavior of electrode potentials was sometimes noted in cultures growing in the basic medium. Oftentimes only one electrode was affected. After a few hours or days the two electrodes would again be in perfect agreement and follow the usual course. The cause of these peculiar variations was not determined.

TEMPERATURE RELATIONS

Comparative thermal relations of these organisms were studied by measuring the diameter of giant colonies at stated temperatures on yeast-extract glucose agar after 14 days of incubation. The cultures were grown in 6-ounce screw-cap bottles which contained a thick layer of agar adjusted to pH 7.0. Needle pricks were used to start the colony on the surface of the substrate. The average diameter of the colonies in millimeters was taken as an index of growth at temperatures between 4° and 36° C.

The results of this study are presented graphically in figure 4. The region of optimum growth under the conditions of this experiment for all of these cultures, except the beet gall organism, occurred at 28° C. For all practical purposes this temperature can also be considered satisfactory for the beet gall organism, since only a slight increase of colony size over 28° was recorded for its observed optimum of 32° and since this difference is within experimental error. It is interesting, physiologically, that this group of bacteria should have similar thermal relations in culture.

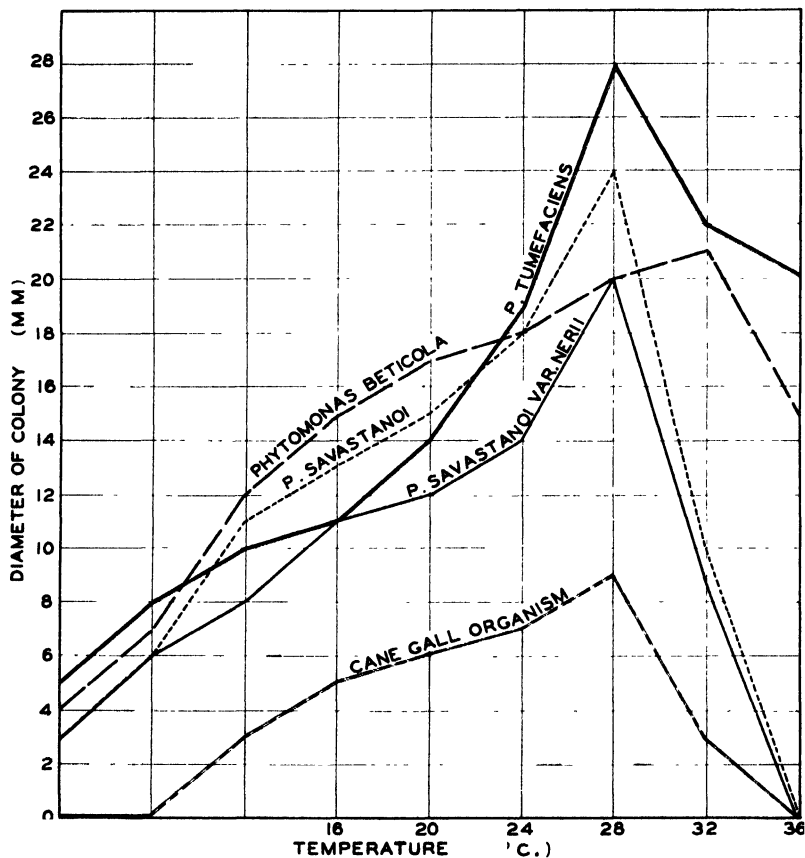


FIGURE 4. —Growth of various gall-forming plant pathogens as measured by colony diameter on yeast-extract-glucose-mineral-salts agar at stated temperatures during 14 days

HYDROGEN-ION RELATIONS

The maximum and the minimum hydrogen-ion concentration at which growth was initiated were studied in the basic medium containing glucose. Sodium hydroxide or hydrochloric acid was added to the media to shift the hydrogen-ion concentration to the limits of growth. The alkaline media were stored over strong alkali to prevent carbon dioxide of the air from changing the initial reaction. This procedure was only partly successful, however, because any

growth produced by the organisms tended to acidify the medium. Only the rough alkaline limits could be determined by this method because the alkaline media gradually shifted toward neutrality and after a few days or a week bacterial growth was invariably initiated in all tubes. No trials were made in a more highly buffered medium.

The results of the studies revealed that the crown gall, cane gall, and olive knot bacteria were similar in the acid regions and were prevented from establishing growth between the ranges of pH 4.2 and 4.4; while the oleander gall organism was unable to establish growth at pH 3.8 to 4.0; and the beet gall organism, at pH 3.6 to 3.8. The cultures were incubated 21 days at 24° C. The alkaline tolerance of the group could only be approximated, as previously explained. Indications up to 1 week pointed to the pH region of 9.5 to 10.5 for the whole group as the limit of alkaline tolerance under the conditions of this experiment. Figure 3 shows the E_h of the sterile medium at various hydrogen-ion concentrations. Doubtless the E_h was an important influence, as well as pH at the limits of growth.

UTILIZATION OF NITROGEN AND CARBON COMPOUNDS

The ability of the gall-forming bacteria to utilize selected sources of nitrogen and carbon in a mineral-salt medium was examined to learn more of the comparative physiology of these organisms and to determine, if possible, any similar or dissimilar metabolic characteristics. These characters were measured by (1) the relative amount of growth produced in the tubes, (2) the change in titratable acidity, and (3) the final hydrogen-ion concentration of the culture.

UTILIZATION OF VARIOUS NITROGENOUS COMPOUNDS

The various media for studying nitrogen utilization were made by substituting the nitrogen compounds listed in table 3 for the yeast extract of the basic medium. Concentrations of 0.5 percent by weight were used in these media except in the case of l-cystine, uric acid, and l-tyrosine which were employed in 0.1 percent concentrations. Traces of available food materials (probably nitrogenous) were detected in the distilled-water supply during preliminary studies. These impurities of the water were removed by redistillation over potassium permanganate.

The comparative growth of these organisms in various nitrogenous media containing respectively various nitrogenous compounds is summarized in table 3. The crown gall organism appeared to use many and diverse, simple and complex compounds while the cane gall organism used the more complex compounds. The other cultures were apparently intermediate. Of the 20 media tested in this experiment 9 were used by all of the organisms. Imide, amide, amino, and organic ammonium nitrogen characterize the compounds utilized by all of the organisms.

The comparative titratable acidity or alkalinity induced by these organisms in various nitrogenous media is also summarized in table 3. Since detailed treatment of the data would require too much space, only the more outstanding variations are noted. The crown gall bacteria produced marked quantities of total alkalinity in urea and l-asparagine media. The cane gall bacteria produced large

TABLE 3.—Growth, hydrogen-ion concentration, and change in titratable acidity produced by five gall-forming bacteria in glucose-mineral-salt media containing various nitrogen sources

Nitrogen source ¹	Growth ² of organism causing—					Control tubes	pH concentration with organism causing—				Control tubes ²	Change in titratable acidity ⁴ with organism causing—					
	Crown gall	Cane gall	Beet gall	Olean-der gall	Olive knot		Crown gall	Cane gall	Beet gall	Olean-der gall		Olive knot	Crown gall	Cane gall	Beet gall	Olean-der gall	Olive knot
Potassium nitrate.....	+++	0	++	+++	++	pH 6.5	7.1	6.4	4.0	4.7	4.1	Cc	-0.05	-0.15	+0.15	+0.25	+0.15
Ammonium nitrate.....	+++	0	++	++	0	6.6	4.7	5.9	3.9	5.3	5.5	7.0	+0.60	+0.30	+0.40	+0.10	+0.10
Ammonium sulphate.....	+++	0	++	++	0	6.8	4.5	6.0	3.8	5.5	5.8	1.00	+0.80	+0.00	+0.10	+0.10	+0.00
Ferrie ammonium citrate.....	+++	0	++	++	0	7.1	7.8	6.0	5.9	7.6	7.7	1.06	+0.14	+0.14	+0.04	+0.36	+0.16
Potassium nitrite.....	+++	0	++	++	0	6.9	6.9	7.3	7.8	8.0	7.7	.40	-0.20	-0.35	-0.10	-0.10	-0.10
Uric acid.....	+++	0	++	++	0	6.7	6.6	3.9	4.7	8.0	6.5	.15	+0.15	+0.05	+0.15	+0.05	+0.15
Urea.....	+++	0	++	++	0	6.2	8.8	7.0	3.3	8.0	9.0	.60	-1.20	-0.35	+0.10	-0.20	+0.10
Dicyandiamide.....	+++	0	++	++	0	7.0	5.1	7.1	7.2	4.2	4.1	.06	+0.24	-0.04	+0.04	+0.04	+0.12
Oxamide.....	+++	0	++	++	0	7.0	4.7	4.7	3.8	4.1	4.5	.10	+0.46	+0.46	+0.76	+0.36	+0.06
Acetamide.....	+++	0	++	++	0	7.0	4.8	6.7	3.0	5.3	4.0	.50	-0.50	+0.00	+0.35	+0.30	+0.15
Succinamide.....	+++	0	++	++	0	7.0	4.4	4.7	3.2	5.3	5.1	1.20	-1.00	+0.80	+0.15	+0.40	+0.20
L-asparagine.....	+++	0	++	++	0	6.8	8.5	4.6	5.3	8.5	8.6	2.30	+0.70	+0.70	+0.30	+0.30	+0.10
Barbital sodium.....	0	0	0	0	0	7.1	6.8	6.6	6.6	4.2	4.5	.40	-0.40	-0.25	-0.40	-0.40	-0.25
Guandine carbonate.....	0	0	0	0	0	6.5	8.2	8.0	8.1	8.0	8.0	.50	+0.10	+0.10	+0.80	+0.50	+0.25
Glycine.....	+++	0	++	++	0	6.9	6.7	8.0	8.0	8.0	8.0	.50	+0.10	+0.15	+0.45	+0.50	+0.10
L-tyrosine.....	+++	0	++	++	0	6.8	3.8	3.4	3.0	3.8	3.5	.20	+0.30	+0.10	+0.35	+0.30	+0.40
L-lysine.....	+++	+++	++	++	++	7.0	4.7	5.0	3.1	4.0	3.8	.36	+0.25	+0.10	+0.80	+0.30	+0.40
D-glutamic acid.....	+++	+++	++	++	++	7.0	6.9	4.8	4.4	4.3	4.3	.15	+0.25	+1.05	+0.15	+0.35	+0.85
Yeast extract.....	+++	+++	++	++	++	7.0	6.4	3.7	3.2	3.6	3.9	.13	+0.17	+0.57	+0.07	+0.47	+0.57
None.....	0	0	0	0	0	7.0	6.7	6.5	6.9	6.9	6.2	.10	.00	.05	.00	.00	+0.10

¹ Neutralized with sodium hydroxide, as explained in the text² Amount of growth indicated as follows: 0, none or trace; ++, slight³ Cubic centimeters of 1% sodium hydroxide necessary to bring 10 cc of the sterile medium to the neutral point of phenolphthalein⁴ In each case the increase (+) or decrease (-) in total acidity compared to the control tubes is recorded in cubic centimeters of 1/50 N sodium hydroxide or hydrochloric acid. Phenolphthalein was used as the indicator.

quantities of acid in succinimide, and d-glutamic acid media. The beet gall bacteria produced considerable acid especially in succinimide and l-asparagine media and notable amounts in several others. The oleander gall and olive knot organisms were similar in most of the compounds tested and resembled the crown gall bacteria in the production of an alkaline reaction from l-asparagine. The only consistent relation among these cultures, when they grew, appeared in the total acidity induced in media made with uric acid, oxamide, l-tyrosine, l-cystine, and yeast extract.

The comparative hydrogen-ion concentrations induced in these various nitrogenous media are summarized in table 3. Some of these media, as already mentioned, supported visible growth of all five cultures. Among such media those made with oxamide, l-tyrosine, and l-cystine became acid with each organism. All the organisms which grew and induced similar reactions, produced acid in media made with acetamide, barbital sodium, ammonium nitrate, and ammonium sulphate. No comparable alkaline reactions were induced. These results should be considered in relation to the total acidity discussed earlier.

Oxidation-reduction intensities of these media were measured before seeding and again after an incubation period of 21 days at 24° C. Detailed treatment of these data is beyond the scope of this paper. As much as 0.05-volt drift was noted in some of the sterile media during the incubation period. The maximum and minimum E_h limits of all of the sterile media lay within $E_h + 0.50$ volt and $+0.39$ volt which represents rather oxidized conditions but which were within the limits of growth in the basic medium. The inability of certain organisms to grow in some of the media cannot be explained by an unfavorable initial oxidation-reduction potential. Yeast-extract-glucose liquid medium, one of the most favorable for growth, started at $E_h + 0.44$ volt. Others ranged from $+0.39$ to $+0.50$ volt. The final electrode potentials of the cultures were commonly within this same range. However, there were some notable exceptions many of which appeared when the culture had a relatively strong alkaline reaction. The consideration of these E_h readings must take into account both the facts (1) that the E_h readings represent the resultant of the oxidation-reduction conditions and the hydrogen-ion concentration, and (2) that the oxidation-reduction conditions become rapidly more oxidizing at the end of the active growth period.

UTILIZATION OF VARIOUS SOURCES OF CARBON

Preparation of the various media employed in the utilization of carbon was made by substituting the carbon compounds listed in table 4 for the glucose of the mineral-salt-yeast-extract basic medium.

The comparative growth of the gall-forming bacteria in media containing various sources of carbon is given in table 4. A trace of growth appeared in the yeast extract which prevented a clear-cut picture of utilization as compared with no utilization of the compound in question. The use of yeast extract as a source of nitrogen was essential for growth of all the organisms studied. It was clear that most of the carbon media tested were available to the needs of the bacteria. The notable exception appeared to be oxalic acid.

TABLE 4.—Growth, hydrogen-ion concentration, and change in titratable acidity produced by five gall-forming bacteria in yeast-extract-mineral-salt liquid media containing various carbon sources

Carbon source ¹	Growth ² of organism causing—					pH concentration with organisms causing—					Change in titratable acidity ⁴ of organism causing—					
	Control					Control tubes 3					Control					
	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot	
L-arabinose.....	++	++	++	++	++	6.3	6.3	4.1	4.2	3	6.1	Cc 0.50	Cc +0.25	Cc +0.70	Cc +0.50	Cc 0.00
D-xylose.....	++	++	++	++	++	6.3	6.3	4.1	4.6	4.6	4.3	0.50	+	+	+	+
D-fructose.....	++	++	++	++	++	6.5	6.5	5.6	4.7	4.7	4.0	0.50	+	+	+	+
D-mannose.....	++	++	++	++	++	6.2	6.2	5.1	4.6	4.4	4.2	0.50	+	+	+	+
D-galactose.....	++	++	++	++	++	6.6	6.6	4.9	3.2	3.8	3.5	0.50	+	+	+	+
D-glucose.....	++	++	++	++	++	6.6	6.2	4.0	3.4	3.8	4.0	0.50	+	+	+	+
Sucrose.....	++	++	++	++	++	6.3	6.3	3.8	3.4	3.4	4.0	0.50	+	+	+	+
Maltose.....	++	++	++	++	++	7.0	7.5	3.7	3.2	3.4	4.0	0.50	+	+	+	+
Lactose.....	++	++	++	++	++	7.2	7.6	4.4	4.5	4.4	8.3	0.50	+	+	+	+
Melzitose.....	++	++	++	++	++	6.8	6.6	4.4	4.2	4.4	8.2	0.50	+	+	+	+
Raffinose.....	++	++	++	++	++	6.7	7.0	3.8	3.9	4.0	8.0	0.50	+	+	+	+
Starch.....	++	++	++	++	++	6.8	6.8	6.8	5.0	5.0	8.0	0.50	+	+	+	+
Inulin.....	++	++	++	++	++	6.5	8.2	8.1	8.1	8.1	8.0	0.50	+	+	+	+
Pectin.....	++	++	++	++	++	6.7	6.7	8.0	8.2	8.1	8.1	0.50	+	+	+	+
Glycerol.....	++	++	++	++	++	7.3	7.0	7.2	6.1	6.1	8.1	0.50	+	+	+	+
Erythritol.....	++	++	++	++	++	6.3	7.5	7.1	5.6	6.3	7.8	0.50	+	+	+	+
Lactosul.....	++	++	++	++	++	7.0	6.6	4.2	3.0	8.1	8.1	0.50	+	+	+	+
Mannitol.....	++	++	++	++	++	7.2	6.9	4.2	3.0	8.1	8.1	0.50	+	+	+	+
Dulcitol.....	++	++	++	++	++	7.3	6.4	4.3	3.1	8.0	8.1	0.50	+	+	+	+
Salicin.....	++	++	++	++	++	7.1	6.2	4.4	3.4	8.0	8.2	0.50	+	+	+	+
Asculin.....	++	++	++	++	++	6.6	6.6	6.7	5.3	8.2	8.2	1.90	+	+	+	+
Phloridan.....	++	++	++	++	++	6.5	6.8	6.7	5.0	8.2	8.2	1.50	+	+	+	+
Calcium gluconate.....	++	++	++	++	++	8.0	9.2	8.8	9.1	9.1	9.4	25	+	+	+	+
Formic acid.....	++	++	++	++	++	8.0	9.2	8.8	9.1	9.1	9.4	25	+	+	+	+
Acetic acid.....	++	++	++	++	++	8.0	9.0	8.0	8.4	8.1	8.8	10	+	+	+	+
Propionic acid.....	++	++	++	++	++	7.3	8.9	8.0	8.4	8.1	8.8	10	+	+	+	+
Glycolic acid.....	++	++	++	++	++	7.3	8.1	8.0	9.1	8.0	9.3	1.00	+	+	+	+
Malonic acid.....	++	++	++	++	++	8.0	8.0	8.0	9.1	8.0	9.3	1.00	+	+	+	+
Oxalic acid.....	++	++	++	++	++	8.0	8.0	8.6	9.3	8.3	8.2	0.50	+	+	+	+
Succinic acid.....	++	++	++	++	++	7.3	8.4	8.6	9.3	8.3	9.2	15	+	+	+	+
Tartaric acid.....	++	++	++	++	++	7.3	8.0	8.1	9.2	9.2	9.1	0.50	+	+	+	+
Malic acid.....	++	++	++	++	++	7.3	8.2	8.1	9.5	9.5	9.4	15	+	+	+	+
Citric acid.....	++	++	++	++	++	7.0	8.2	8.1	9.2	9.1	9.0	10	+	+	+	+
None (yeast extract only) ⁵	++	++	++	++	++	7.0	8.2	8.0	9.4	9.4	9.1	10	+	+	+	+

¹ Neutralized with sodium hydroxide as explained in the text.² Amount of growth indicated as follows: 0, none; +, slight.³ Cubic centimeters of 1/20 N sodium hydroxide necessary to bring the sterile medium to the neutral point of phenolphthalein.⁴ In each case the increase (+) or decrease (−) in total acidity compared to the control tubes is recorded in cubic centimeters of 1/20 N sodium hydroxide or hydrochloric acid.

Phenolphthalein was used as the indicator.

Of the 35 compounds tested, 24 were undoubtedly used by all of the organisms to some extent, as indicated by the turbidity produced over and above that of the control tubes.

The comparative titratable acidity or alkalinity developed in these media are also summarized in table 4. Consistent production of approximately the same amount of acid or alkaline materials by all these organisms residing in the same medium was not observed except in limited instances. Each of the organisms yielded values approximately similar to those induced by the others in media containing the following sources of carbon: Melezitose, raffinose, starch, inulin, pectin, glycerol, and glycollic acid. The total acidity values of these media differed slightly among themselves.

The comparative hydrogen-ion concentrations induced by these organisms in the various carbon-containing media are shown in table 4. Where there was little or no growth, there was often no important change. At least slight acid reactions were induced by all of the organisms in d-fructose, d-glucose, d-mannose, and d-galactose. Varying acid to neutral reactions were induced by all of the organisms in l-xylose, aesculin, glycerol, phloridzin, and mannitol. Alkaline reactions were induced by all of the organisms in melezitose, starch, yeast extract, and in all of the sodium salts of the organic acids studied. The alkalinity developing from the latter compounds is probably explained by release of the alkaline cation following utilization of the organic radical of the molecule. A slight alkalinity was induced in the yeast extract medium which contained no added source of carbon. In media such as that containing oxalic acid, only a slight growth was induced accompanied by an alkaline reaction, which apparently came from the yeast extract. For the most part the crown gall organism produced little, if any, acid from the various sources of carbon. The cane gall and beet gall organisms produced acid from several of the sugars while the oleander and olive knot organisms had a tendency to produce an alkaline reaction. The classification used is highly artificial and is presented merely for convenience.

The average E_h limits of the sterile control tubes fell between $E_h + 0.48$ and $E_h + 0.43$ volt except in sodium formate which was $+0.17$ volt. No appreciable drift was noted during the incubation period (21 days at 24°C.) in any of the sterile media. The media containing various carbon sources was found to be poised at a satisfactory potential for growth of these bacteria at the beginning of the experiments. In the case of the crown gall bacteria slight reducing conditions were found in all media except that containing phloridzin. With regard to the remaining pathogens, low E_h values were found in most of the carbon-containing media except where strong acid reactions developed. A consideration of these E_h readings must take into account the pH relationships mentioned earlier, the time-potential curves mentioned later, and the changes incurred by cessation of growth. Detailed treatment of these results are beyond the scope of this paper.

Parts of earlier work by Brown (4), Riker (14), C. O. Smith (18), and E. F. Smith (19) have been repeated and confirmed by the present studies in which the various organisms have been examined in parallel cultures.

Quantitative fermentation studies of reducing sugars were made according to the method of Stiles et al. (22). One-half percent sugar solutions, in 10-cc lots were used as noted previously.

TABLE 5.—*Action of certain cell-stimulating plant pathogens on reducing sugars as measured by quantitative determinations of reducing substances remaining in the cultures after fermentation for 21 days at 24° C.*

Carbon source	Sugar fermented ¹ by organisms causing -				
	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot
	Percent	Percent	Percent	Percent	Percent
d-glucose	31	29	55	49	37
d-galactose	35	22	65	41	35
d-mannose	42	25	80	20	34
d-fructose	67	40	89	30	59
l-xylose	35	26	73	9	50
l-arabinose	62	29	68	11	46
Sucrose	41	23	79	31	2
Maltose	47	50	85	73	64
Lactose	16	20	24	20	20

¹ Control tubes analyzed as 100 percent

² Slight fermentation was probable but not sufficiently abundant to measure successfully

The results of analysis of total reducing substances in the culture after the usual incubation period (21 days at 24° C.) are summarized in table 5. Maltose and d-fructose were fermented in the largest quantities by most of the organisms studied. No utilization of lactose could be found in the case of cane gall, oleander tubercle, or the olive knot bacteria. The oleander organism fermented l-xylose and l-arabinose only slightly; sucrose was scarcely used by the olive knot organism. Moderate turbidity was noted in all these sugar solutions. It should be mentioned that large-bore test tubes gave a higher percentage of fermentation than did small tubes, although the same quantities of media were used. All the tubes were capped with tin foil to prevent evaporation; consequently, the degree of fermentation was probably less than it would have been in fully aerated cultures. However, the fact that certain sugars were used appears clearly in these results. Their utilization might be questioned in some cases when only acid production was considered.

OXIDATION-REDUCTION STUDIES IN LIQUID CULTURES

Comparative time-potential measurements were made in yeast-extract-glucose-mineral-salts liquid media, the technic used being similar to that of Allyn and Baldwin (2). The platinum electrodes were sterilized in distilled water and gave a potential of approximately +0.48 volt at pH 7.2 in the sterile basic medium at the beginning of the experiment. Daily readings were made on undisturbed cultures. The results are plotted in figure 2. The final values of the electrode potentials represent an average reading of four electrodes since each experiment was made in duplicate. Although the work was repeated three times, only the results of one representative experiment are shown. The results in the other two trials were similar but are omitted because of their volume.

Characteristic drop in the E_h value was observed in all the cultures during the first 3 days. The velocity of change was apparently governed by the rate of growth since the cane gall bacteria were

consistently slow as compared with the other organisms. No correction was made for the pH effect. The influence of pH upon the E_h reading has already been examined (fig. 3) in the basic medium employed. It is interesting to observe that, when the bacteria formed acid which alone would raise the E_h values, nevertheless, the metabolism of the organisms lowered the E_h readings. Even though these cultures apparently behaved alike in this experiment, it should be noted that other organisms (2, 7, 17) show similar curves. However, it appears altogether likely that certain organisms, when capable of growing in plant tissue, may exert a distinctly reducing effect. Further studies in this field were beyond the scope of this paper.

DISCUSSION

Some differences in the comparative physiology of the above-mentioned organisms (tables 1 and 2) appeared during identification, although the oleander and olive knot cultures were closely related. Among these five cultures, that from beet gall exhibited the widest differences in that it was pigmented, caused slight digestion of casein, slight hydrolysis of starch, and slight liquefaction of gelatin. In temperature studies its optimum of 32° C. was several degrees above that of the other cultures. All the cultures, except those of the oleander and olive knot, were different from each other in litmus milk. All of the cultures were parasitic on the hosts from which they were isolated. Only the crown gall organism infected all the hosts employed.

A suggestion of nitrogen fixation similar to that reported earlier (reviewed by Riker and Berge (15) was found in preliminary experiments, but later a small amount of utilizable material was detected in the distilled water. In further experiments the water was redistilled over potassium permanganate which removed the utilizable material. In the nitrogen-free (except atmospheric nitrogen) media no growth occurred, which shows the inability of these organisms to fix much, if any, atmospheric nitrogen in these cultural media. Consequently, reports that nitrogen fixation is common in laboratory media may be open to question, and suggestions concerning the cause of cell stimulation based on nitrogen fixation seem to have an inadequate experimental background.

In the studies on the utilization by these organisms of materials containing carbon and nitrogen, valid measurements on which they could be compared appeared to be growth, hydrogen-ion concentration induced, and change in titratable acidity. Titratable acidity was commonly, but not always, reflected in the reaction. Change of oxidation-reduction potentials appeared of doubtful value as a measure of carbon or nitrogen utilization. Since all the organisms studied induce plant overgrowths, and although the possibility is recognized that they may induce the stimulation in different ways, it would seem that similarities in their physiology are of more importance than dissimilarities when considered in relation to the general question of atypical and pathological multiplication of plant cells. Consequently emphasis is placed on characters which the organisms have in common. The following list shows the similarities in growth and reaction induced by these organisms in media with various sources of carbon and nitrogen. The crown gall and beet gall organisms showed the least similarities encountered, whereas the oleander and

olive knot organisms showed the greatest similarities in that they grew and produced comparable reactions in 46 of the 54 compounds tested.

<i>All organisms studied</i>		
Starch	Oxalic acid	Dulcitol
Pectin	Citric acid	Citric acid
Phloridzin	Ferric ammonium citrate	Potassium nitrate
Formic acid	Uric acid	Acetamide
Acetic acid	l-asparagine	Succinimide
Propionic acid		d-glutamic acid
Glycollic acid	<i>Cane gall and beet gall organisms</i> ⁴	Yeast extract
Malonic acid		<i>Beet gall and olive knot organisms</i> ⁴
Succinic acid	l-arabinose	l-xylose
Tartaric acid	l-xylose	d-fructose
Malic acid	l-rhamnose	d-galactose
Oxamide	d-mannose	d-glucose
l-tyrosine	d-galactose	Raffinose
l-cystine	d-glucose	Inulin
	Melezitose	Lactositol
<i>Crown gall and cane gall organisms</i> ⁴	Inulin	Erythritol
Sucrose	Ferric ammonium citrate	Dulcitol
Maltose	Uric acid	Citric acid
Raffinose	Succinimide	Potassium nitrate
Dextrin	l-asparagine	Acetamide
Glycerol	d-glutamic acid	Succinimide
Lactositol	Yeast extract	d-glutamic acid
Dulcitol	<i>Cane gall and oleander gall organisms</i> ⁴	Yeast extract
Aesculin		<i>Oleander and olive knot organisms</i> ⁴
Calcium gluconate	d-fructose	Rhamnose
<i>Crown gall and beet gall organism</i> ⁴	d-galactose	d-fructose
Lactose	d-glucose	d-galactose
Erythritol	Inulin	d-glucose
Oxalic acid	Aesculin	Sucrose
Citric acid	Calcium gluconate	Maltose
	Succinimide	Lactose
<i>Crown gall and oleander gall organism</i> ⁴	d-glutamic acid	Melezitose
	Yeast extract	Raffinose
	<i>Cane gall and olive knot organisms</i> ⁴	Inulin
d-mannose	l-xylose	Dextrin
Sucrose	d-fructose	Glycerol
Glycerol	d-galactose	Erythritol
Erythritol	d-glucose	Lactositol
Mannitol	Inulin	Mannitol
Calcium gluconate	Glycerol	Dulcitol
Oxalic acid	Aesculin	Salicin
Citric acid	Calcium gluconate	Aesculin
Ferric ammonium citrate	Succinimide	Calcium gluconate
Uric acid	d-glutamic acid	Citric acid
l-asparagine	Yeast extract	Potassium nitrate
<i>Crown gall and olive knot organisms</i> ⁴	<i>Beet gall and oleander gall organisms</i> ⁴	Ferric ammonium citrate
l-arabinose		Uric acid
l-rhamnose	d-fructose	Urea
Glycerol	d-galactose	Dicyandiamide
Erythritol	d-glucose	Acetamide
Mannitol	Raffinose	Succinimide
Aesculin	Inulin	l-asparagine
Calcium gluconate	Erythritol	Barbital-sodium
	Lactositol	Glycine
		d-glutamic acid
		Yeast extract

⁴ In addition to compounds used by all organisms studied.

Certain reactions induced are particularly noteworthy. Utilization of the sodium salts of the organic acids together with the production of alkali was found with all the cultures. Similarly, oxamide, l-tyrosine and l-cystine were used by all the cultures, but with the formation of acid. With many other substances varying degrees of differences in the reaction induced indicate that neither simple acidity nor simple alkalinity should be considered the direct factors of host-cell stimulation.

Although the production of an acid or alkaline reaction in sugar media is a common indication of its utilization, it is unreliable if neutral products are formed. Therefore, quantitative determinations of reducing substances remaining in the cultures after fermentation were made and are given in table 5. Lactose was utilized sparsely by all of the cultures, and sucrose was not fermented to any extent by the olive knot organism. l-xylose was not appreciably utilized by the oleander gall organism. It would seem that lactose and l-xylose could be included with many other substances to be set aside during studies of materials that may contribute to the development of stimulating factors until certain others have received more attention.

The hydrogen-ion activities at levels in which growth does not occur in the basic medium appears much more significant when the interrelationship of pH and E_h are considered together (fig. 3). Shifting the pH from 4.0 to 10.0 with sodium hydroxide involved a change in E_h of nearly 312 millivolts in the basic medium. This range of voltage is narrow as compared with observed ranges caused by cell proliferation in undisturbed media (fig. 2). Under these conditions the E_h range of growth was approximately 600 millivolts. If it is true that the alkaline limit of growth lies in the region of pH 10.0 than the E_h of the basic medium (+0.265 volt) approaches an optimum at this level (fig. 2), and the pH then becomes an inhibiting factor of growth. In the acid region near pH 4.0 both the hydrogen-ion activity and the E_h (+0.556 volt) approached the limit of growth, and either one or the combination may, therefore, be the limiting factor. In limited unpublished experiments, an unsuccessful attempt was made to separate these two apparently limiting factors by poisoning the basic medium at stated E_h -pH levels by using common oxidizing or reducing agents in the medium. Certain indications appeared suggesting that the limiting oxidation-reduction range of this group of cultures lay near +0.6 volt on the oxidized side of the normal hydrogen electrode and near -0.3 volt on the reduced side, which suggests that pH was the limiting factor in the acid region. Furthermore, the concentration of the reagents used to reach these physical limits had undesirable effects upon the medium. These studies indicate the great practical importance of considering E_h as well as pH for the growth of these pathogenic microorganisms in culture. No doubt it is very important also for the growth of microorganisms in the plant and has a bearing on both susceptibility and resistance.

That the oxidation-reduction potential of a medium may play a significant role in the ability of aerobic organisms to grow in certain media has been shown by Allyn and Baldwin (1, 2). The inability of an organism to utilize certain nutrients may, therefore, be a function of E_h . In order to determine whether this factor, as well as pH, was operating to prevent growth in the media used, tests were made

on the oxidation-reduction potentials developed in the media containing various sources of carbon and nitrogen before seeding and after incubation. These results, while not reported in detail, indicated that none of these media were beyond the range of E_h suitable for the growth of these organisms. Final potential readings were of greater oxidation intensity than the final readings of the time-potential measurements of undisturbed liquid cultures in the basic glucose medium. These results are attributed largely to cessation of growth and to auto-oxidation before the final readings were taken. In accord with figure 3, the production of an acid reaction in the medium seemed to be correlated with relatively strong oxidizing potentials, and, conversely, relatively strong reducing potentials were apparently correlated with alkaline reactions. Sodium formate gave the most reducing readings of all of the compounds in this study both before and after incubation. In this case the difference in E_h was clearly more than could be accounted for on the basis of pH differences.

Studies on electrode potentials developed in liquid cultures in relation to time (fig. 2) showed, for all of the organisms, increased reducing potentials with increase of time, regardless of hydrogen-ion concentration. A study of the nature of the factors causing this reduction was not undertaken. Since other unrelated organisms show similar results, this characteristic is not peculiar to the gall-forming group. If these cell-stimulating bacteria bring about a relatively reduced potential within the host tissue as was found in culture, this change would probably influence the unknown factors that stimulate growth. However, Hendrickson et al. (7) and Sagen et al. (17) found that the reducing action of nonpathogenic cultures was apparently similar to that of pathogenic cultures.

It appears in the data presented not only that physiological similarities and dissimilarities among certain cell-stimulating bacteria have been clarified, but also that there are certain implications concerning factors (reviewed (15)) possibly important in relation to atypical and pathological multiplication of cells.

SUMMARY

Cross inoculations, with small pieces of gall tissue and with single-cell cultures of *Phytoplasma tumefaciens*, *P. beticola*, *P. savastanoi* var. *nerii*, *P. savastanoi*, and an unnamed organism causing raspberry cane gall, made on tomato, garden beet, oleander, olive, and raspberry showed (1) that *P. tumefaciens* was pathogenic on all of the hosts used, (2) that *P. savastanoi* var. *nerii* was pathogenic on the oleander and olive, and (3) that each of the other organisms was pathogenic only on the host from which it was isolated.

Routine physiological studies on certain common bacteriological media showed that each of these bacterial organisms gave characteristic growth reactions which were distinctive for each organism, except the oleander and olive knot bacteria which showed similar cultural characters in all these media.

The optimum temperature range for colony diameter on agar was about 28° C. for all of these micro-organisms except *Phytoplasma beticola* which produced its largest colonies at 32°.

The hydrogen-ion concentration at which growth was prevented in liquid culture was found to vary from pH 3.6 to 4.4 in the acid range and approximately 9.5 to 10.5 in the alkaline range.

Comparative utilization of several types of compounds indicated that oxamide, l-tyrosine and l-cystine were the only sources of nitrogen used in which all of the organisms distinctly produced similar reactions. Hydrogen-ion determinations were all in the acid range.

Comparative utilization of several types of compounds containing carbon indicated that starch, pectin, phloridzin, and the sodium salts of formic, acetic, propionic, glycollic, malonic, succinic, tartaric, and malic acids were the only sources of carbon used in which all of the organisms distinctly produced similar reactions. Hydrogen-ion determinations were all in the alkaline range.

Supplementing these studies, quantitative determinations were made of reducing substances remaining after fermentation in the cultures containing the reducing sugars. Some of the cultures showed similar, and other unequal, utilization. In some cases little or no loss of sugar was detected by the analyses, although sugar fermentation was indicated by turbidity and by change of reaction.

The inability of certain of these organisms to utilize several of the compounds containing carbon or nitrogen was not caused by unfavorable oxidation-reduction intensities of these media at hydrogen-ion concentrations approaching neutrality.

Hydrogen-ion concentration of the sterile yeast-extract-glucose-mineral-salts liquid medium was found to have a marked influence on the oxidation-reduction intensity of this medium. Strong acid reactions were accompanied by relatively strong oxidizing potentials, either or both of which may have limiting or at least unfavorable influences on growth in the extreme acid region.

Oxidation-reduction-potential measurements made at frequent time intervals with various growing cultures in a yeast-extract medium showed that all these organisms produced relatively strong reducing potentials in undisturbed liquid cultures. This occurred in opposition to the oxidizing action of acid metabolic products.

The similarities of these organisms are discussed in relation to their comparative physiology and its bearing on certain working hypotheses for the cause of atypical and pathological multiplication of plant cells.

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OVARIAN DEVELOPMENT IN CALVES ¹

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INTRODUCTION

The presence of follicles of approximately mature size in calves 5 to 12 weeks of age, reported by Heitz and confirmed by Kappeli and Küpfer, is referred to by Hammond.² The last-named writer states that the age of puberty is probably reached at about 9 months and comments upon the length of the interval from the appearance of large follicles to puberty.

It has been shown for the rabbit and the pig respectively (Hertz and Hisaw,³ and Casida⁴) that the immature female does not react macroscopically to gonad-stimulating extracts of the pituitary until an age is reached when vesicular follicles are present in the ovary. The observations mentioned above indicate that very young calves may be suitable for experimentation on the effects of various gonad-stimulating extracts on the bovine ovary; furthermore, such animals are desirable for experimental work for economic reasons. A study of the degree of follicular development in heifer calves which had been slaughtered for veal was accordingly made to determine the suitability of such animals for experimentation and also to furnish data on normal variation in ovarian development.

MATERIAL AND METHODS

Genitalia from 190 heifer calves showing Holstein-Friesian color markings and from 83 calves of various other breeds (many of Guernsey coloring) were collected⁵ and the weight of the carcass (dressed weight) obtained for each animal. These collections were made during February and March 1934; as far as could be determined the calves were from southern Wisconsin and northeastern Iowa.

The collected genitalia were brought to the laboratory, where determinations were made of the following characters of the right and left ovaries separately: Weight to the nearest decigram, number and diameter of visible vesicular follicles, number of blood follicles, and length, width, and depth of the ovary to the nearest millimeter. The external diameter of one uterine horn was also obtained at a level just above the external bifurcation.

Analytical balances were used in weighing the ovaries, and the follicle counts and measurements were made with a device similar to an egg candler, which passed a strong beam of light through the

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² HAMMOND, J. *THE PHYSIOLOGY OF REPRODUCTION IN THE COW*. 226 pp., illus. London. 1927.

³ HERTZ, R., and HISAW, F. L. EFFECTS OF FOLLICLE-STIMULATING AND LUTEINIZING PITUITARY EXTRACTS ON THE OVARIES OF THE INFANTILE AND JUVENILE RABBIT. *Amer. Jour. Physiol.* 108: 1-13, illus. 1934.

⁴ CASIDA, L. E. PREPUBERAL DEVELOPMENT OF THE PIG OVARY AND ITS RELATION TO STIMULATION WITH GONADOTROPIC HORMONES. *Anal. Rec.* 61: 389-396. 1935.

⁵ The Oscar Mayer Packing Co., Madison, Wis., made possible the collection of this material.

ovary. The measurements were made with a vernier caliper. External diameters of the follicles were readily obtained if the follicles were protruding from the ovarian surface, but if the follicles were embedded it was necessary to measure the diameter of the translucent surface area produced by the follicular fluid. The dimensions of the ovary are those of the body only and do not include follicles which protruded markedly. The classification of blood follicles comprises a series ranging from large follicles which showed excessive vascularity and congestion in the follicular wall, with slight hemorrhage into the follicular liquor, to those approximately 1 mm in diameter, which appeared macroscopically to contain a bluish-red coagulum.

In the study of the data, calculations were made of the mean (M), standard deviation (σ), standard deviation of the mean (σ_M) or standard error, and simple correlation (r). Tests of the significance of the difference between means were based on Fisher's tables of the probability (P) distribution of t and x for different numbers of observations (N).⁶ The significance of simple correlation coefficients, likewise, was tested by Fisher's table of values of the correlation coefficient for different levels of significance. The probability of equaling or exceeding by chance the different values of the constants was the criterion used for deciding upon their statistical significance. The significance of the difference between correlation coefficients was obtained by converting the values of r into z (Fisher's table of r for values of z) and then comparing the difference between the values of the two z 's with the standard error of their difference. If the calculated value had a probability of chance occurrence of 0.05 or less it was considered significant, meaning that a figure of that value or greater would, on an average, occur 5 percent of the time by chance. When the figure was so large that the probability of occurring by chance was reduced to 1 percent, it was then considered to be statistically highly significant. These two points (0.05 and 0.01 of the normal probability curve) are approximately equal to 2 and 2½ times the standard deviation, respectively.

PRESENTATION OF DATA

A preliminary study showed that practically all of the ovaries contained vesicular follicles. Of the 190 Holstein calves, 98 percent had follicles 1 mm or more in diameter; 89 percent, follicles 4 mm or more in diameter; 41 percent, follicles 10 mm or more in diameter; and 3 percent, follicles as large as 13 mm in diameter. These calves ranged in dressed weight from 52 to 138 pounds. Considering the dressing percentage 65 (as estimated from records of the packing house), the live weight of these heifers was 80 to 212 pounds. This weight range, according to growth data (Brody et al.),⁷ would indicate that the calves ranged from new-born to 14 weeks of age.

The mean dressed weight of the calves of other breeds was distinctly less than that of the Holstein calves (table 1). When the

⁶ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London 1930.

⁷ BRODY, S., and others. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO DOMESTIC ANIMALS. Mo. Agr. Expt. Sta. Research Bull. 96, 182 pp., illus. 1926.

right and left ovaries were considered together as a unit (total ovarian complement) in these two groups, the mean values for all of the ovarian characters, except one (frequency of follicles 1-3 mm in diameter), were numerically greater in the other breeds than in the Holsteins. None of these mean differences, however, was statistically significant. The three characters for which the value of *P* was the lowest (chance difference least likely) were ovarian weight, frequency of follicles 4-13 mm in diameter, and volume of follicles 4-13 mm in diameter. Although the calves of other breeds were distinctly lighter in weight than the Holsteins and showed even greater ovarian development, the nonestimable effect of the breed variability in the former group makes any direct comparison with Holsteins on such a common basis as weight or estimated age seem unjustifiable.

TABLE 1.—Comparison of calves of Holstein appearance with calves of various other breeds

Item	Holstein $M \pm \sigma M$	Other breeds $M \pm \sigma M$	Probability of mean difference
Calves..... number	190	83	
Dressed weight..... pounds	88.6 \pm 1.1	82.8 \pm 1.8	<0.01
Uterine diameter..... millimeters	10.43 \pm .14	10.59 \pm .17	.47
Ovarian weight..... grams	1.90 \pm .09	2.15 \pm .15	.14
Frequency of follicles of different diameters ¹			
1 to 3 mm.....	36.8 \pm 3.6	35.1 \pm 6.0	.88
4 to 6 mm.....	3.34 \pm .30	3.99 \pm .44	.22
7 to 9 mm.....	1.05 \pm .10	1.29 \pm .14	.16
10 to 13 mm.....	.46 \pm .04	.55 \pm .07	.26
4 to 13 mm.....	4.86 \pm .33	5.83 \pm .45	.09
Total volume of follicles of different diameters ^{1,2}			
4 to 6 mm..... cubic millimeters	193 \pm 17	234 \pm 22	.14
7 to 9 mm..... do.....	267 \pm 25	316 \pm 34	.24
10 to 13 mm..... do.....	307 \pm 32	339 \pm 44	.50
4 to 13 mm..... do.....	764 \pm 42	888 \pm 55	.08
Frequency of blood follicles.....	1.04 \pm .12	1.34 \pm .32	.36

¹ Combined right and left ovaries are considered as a unit for all ovarian characters in this table.

² No attempt was made to calculate total volume of follicles 1-3 mm in diameter because of inaccuracy in measurement of the smallest follicles.

Inasmuch as the Holstein calves represented a considerable range in age, as judged by the weight criterion, the association between dressed weight and the various ovarian characters should give some indication as to the type of morphological change which takes place in the ovaries during the first few weeks of postnatal life. Figure 1 shows this in terms of simple correlations. Of the 5 ovarian characters studied, only 3 (weight, frequency of follicles 4-13 mm in diameter, and volume of follicles 4-13 mm in diameter) were significantly correlated with dressed weight. The correlation between dressed weight and volume of follicles 4-13 mm in diameter was significantly higher than that between dressed weight and either of the other characters. Progressive ovarian development is the expression of physiological stimuli, even before puberty; therefore the best indices of the state of ovarian environment are weight of the ovaries and number and total volume of larger follicles, and the best index of all is the total volume of follicles 4-13 mm in diameter.

The mean total volume of follicles 4-13 mm in diameter is determined by the diameter and number of follicles in all calves and by the proportion of calves which have follicles of this size. The Holstein calves were divided into two weight groups (52-86 pounds and 87-138 pounds) of approximately equal frequency (table 2). Many

calves in each group had follicles at least as large as 4 mm in diameter, but the proportion of calves which had follicles of that minimum size was greater by 17 percent in the group of heavier animals. There was also a greater percentage of the heavier calves which had follicles approaching 13 mm in diameter; in addition there was a greater proportion of these heavier calves with more than one large follicle. The percentage of calves having more than 50 follicles less than 4 mm in diameter and also the percentage having blood follicles are very similar in the light and heavy groups. The similarity of the two groups in this last respect agrees with the insignificant correlation between dressed weight and frequency of follicles 1-3 mm in diameter and frequency of blood follicles (fig. 1).

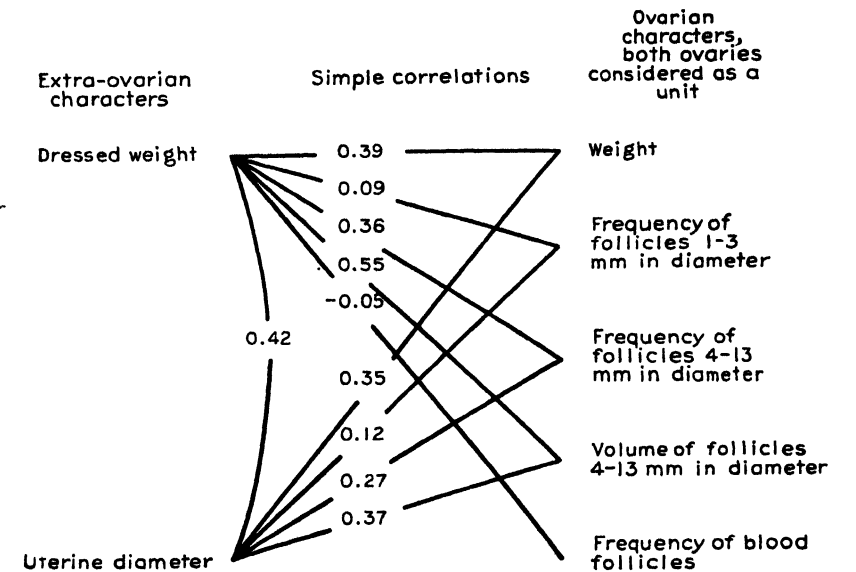


FIGURE 1.—Association among ovarian and extra-ovarian characters in 190 calves of Holstein appearance. Significant correlation=0.15; highly significant=0.18.

TABLE 2.—Ovarian characters as related to dressed weight of calves of Holstein appearance

Calves having—	Percentage of calves of indicated dressed weight having character described	
	91 calves of 52 to 86 pounds	99 calves of 87 to 138 pounds
Visible follicles (any size)	97. 80	98. 99
Follicles over 3 mm in diameter	80. 22	96. 97
Follicles over 6 mm in diameter	61. 53	87. 88
Follicles over 9 mm in diameter	25. 26	54. 54
Follicles 4 to 6, 7 to 9, and 10 to 13 mm in diameter	7. 69	28. 28
More than 1 follicle over 9 mm in diameter	0. 00	8. 08
More than 50 follicles less than 4 mm in diameter	24. 20	22. 22
Blood follicles	50. 54	48. 48

The diameter of the uterine horn was used as a measure of the amount of stimulation being exerted upon the uterus. The significant association between uterine diameter and dressed weight (fig. 1) is interpreted as being, in large part, due to ovarian development, which itself was highly correlated with dressed weight. Significant correlations were obtained between uterine diameter and ovarian weight, and number and volume of follicles 4–13 mm in diameter. Inasmuch as follicular hormone is an important factor in uterine development these correlations suggest that the follicular apparatus in the immature calf is increasing with age not only in magnitude but also in physiological activity, as shown by growth and development in the genital tract.

A comparison was made next of the various ovarian characters in the right and left ovaries respectively. While the situation in the two breed groups of calves was not identical, in the main it was very similar. In the Holsteins consistently higher values were found for the various characters of the right ovaries than for those of the left ovaries with the exception of frequency of blood follicles (table 3). In the calves of other breeds the same was true for the weight and dimensions of the ovary and for the volume of follicles 7–13 mm in diameter. The mean total follicular volume of follicles 4–13 mm in diameter is significantly greater in the right ovaries of both groups, and the mean weight of the right ovary was greater than that of the left in the Holsteins.⁸

TABLE 3.—*Comparison between dimensions and character of right and left ovaries of calves of Holstein appearance and calves of other breeds*

Item	Holstein		Other breeds	
	Right $M \pm \sigma_M$	Left $M \pm \sigma_M$	Right $M \pm \sigma_M$	Left $M \pm \sigma_M$
Ovaries.....number.....	190	190	83	83
Dimensions.....				
Length.....millimeters.....	20.08 \pm 0.28	19.42 \pm 0.30	20.66 \pm 0.44	19.71 \pm 0.43
Width.....do.....	9.75 \pm .23	9.22 \pm .21	10.31 \pm .32	10.23 \pm .36
Depth.....do.....	6.83 \pm .18	6.55 \pm .16	7.47 \pm .25	7.40 \pm .19
Weight.....grams.....	1.01 \pm .05	.89 \pm .04	1.12 \pm .08	1.03 \pm .08
Frequency of follicles of different diameters:				
1 to 3 mm.....	18.9 \pm 1.9	17.9 \pm 1.8	16.7 \pm 2.4	18.6 \pm 2.7
4 to 6 mm.....	1.79 \pm .18	1.55 \pm .16	1.81 \pm .21	2.18 \pm .28
7 to 9 mm.....	.57 \pm .07	.50 \pm .06	.74 \pm .08	.55 \pm .11
10 to 13 mm.....	.28 \pm .03	.17 \pm .03	.34 \pm .06	.22 \pm .05
4 to 13 mm.....	2.64 \pm .19	2.22 \pm .17	2.88 \pm .24	2.95 \pm .29
Volume of follicles of different diameters:				
4 to 6 mm.....cubic millimeters.....	104 \pm 10	88 \pm 9	111 \pm 12	122 \pm 14
7 to 9 mm.....do.....	148 \pm 19	118 \pm 14	184 \pm 22	131 \pm 25
10 to 13 mm.....do.....	194 \pm 24	116 \pm 20	208 \pm 36	131 \pm 29
4 to 13 mm.....do.....	447 \pm 32	317 \pm 25	504 \pm 43	384 \pm 40
Frequency of blood follicles.....	.49 \pm .07	.55 \pm .08	.69 \pm .18	.65 \pm .16

Significant correlations were found between the right and left ovaries of the Holstein calves for the following characters: Frequency of blood follicles, frequency of follicles 4–13 mm in diameter, weight of the ovary, and frequency of follicles 1–3 mm in diameter (table 4). Although the two ovaries showed association in each of these characters, there were significant differences in the values of the correlation coefficients; the items are listed in the preceding sentence in the

⁸ The curves for the distribution of each of the ovarian characters are asymmetrical. Although the type of asymmetry is similar and N is the same for the right and left ovaries, there may be some question as to the validity of this test for significance of the difference. Corroboration of all the differences between the right and left noted in the text was obtained by use of the point binomial, which showed a significantly excessive occurrence of greater values for the characters in the right than in the left ovary.

ascending order of their importance. The volume of follicles 4-13 mm in diameter showed no correlation of significance between the right and left ovaries.

Tables 5 and 6 are presented for the purpose of placing on record the data on normal variation in ovaries of calves of veal size and normal values of ovarian characters for calves of different weights. In addition, they show the reasonably consistent difference between the mean values of the ovarian characters in the right and left ovaries of the Holstein calves when divided into classes based on dressed weight of the calf and the combined weight of the two ovaries.

TABLE 4.—Comparison of the weight and characters of the right and left ovaries in 190 calves of Holstein appearance

Ovarian character	Right ¹ ovary	Left ¹ ovary	Relation between right and left ovary	
			Probability of mean difference	Simple correlation
Weight.....gram	1.01	0.89	< 0.01	0.74
Frequency of follicles 1 to 3 mm in diameter.....	18.9	17.9	.15-.16	.93
Frequency of follicles 4 to 13 mm in diameter.....	2.64	2.22	.08-.09	.60
Volume of follicles 4 to 13 mm in diameter.....cubic millimeter	447	317	< .01	.05
Frequency of blood follicles.....	.49	.55	.40-.50	.37

¹ Refer to table 3 for σ_M of ovarian characters.

TABLE 5.—Weight and characters of right and left ovaries of calves of Holstein appearance as related to dressed weight of calf

Dressed weight (pounds)	Calves	Weight of—		Frequency of follicles 4 to 13 mm in diameter		Volume of follicles 4 to 13 mm in diameter,	
		Right ovary $M \pm \sigma_M$	Left ovary $M \pm \sigma_M$	Right ovary $M \pm \sigma_M$	Left ovary $M \pm \sigma_M$	Right ovary $M \pm \sigma_M$	Left ovary $M \pm \sigma_M$
	Number	Grams	Grams	Number	Number	Cubic millimeters	Cubic millimeters
52 to 72.....	27	0.65±0.09	0.53±0.08	1.5±0.3	1.0±0.2	237±61	87±24
73 to 80.....	37	.80±.11	.81±.08	2.1±.4	1.7±.3	303±81	271±48
81 to 88.....	39	.95±.10	.80±.08	2.4±.3	1.7±.3	401±55	254±50
89 to 96.....	31	1.05±.09	.90±.09	2.8±.4	2.7±.4	491±59	336±66
97 to 104.....	22	1.07±.14	1.10±.15	3.3±.9	2.7±.6	480±89	573±94
105 to 112.....	23	1.52±.17	1.23±.19	3.6±.5	3.5±.6	687±35	369±44
113 to 138.....	11	1.51±.16	1.20±.20	4.4±.7	4.0±.8	918±130	595±150

TABLE 6.—Weight and characters of right and left ovaries of calves of Holstein appearance as related to weight of the two ovaries as a unit

Weight of both ovaries (grams)	Calves	Weight of—		Frequency of follicles 1-3 mm in diameter		Frequency of follicles 4-13 mm in diameter		Volume of follicles 4-13 mm in diameter	
		Right ovary $M \pm \sigma_M$	Left ovary $M \pm \sigma_M$	Right ovary $M \pm \sigma_M$	Left ovary $M \pm \sigma_M$	Right ovary $M \pm \sigma_M$	Left ovary $M \pm \sigma_M$	Right ovary $M \pm \sigma_M$	Left ovary $M \pm \sigma_M$
	Number	Grams	Grams	Number	Number	Number	Number	Cubic millimeters	Cubic millimeters
0.2 to 0.7.....	25	0.29±0.02	0.26±0.02	3.2±1.1	2.8±1.0	0.5±0.2	0.4±0.1	43±16	26±10
0.8 to 1.3.....	39	.52±.03	.51±.03	7.0±1.3	7.2±1.6	1.2±.2	1.5±.3	243±47	245±41
1.4 to 1.9.....	62	.86±.04	.81±.04	12.1±.5	9.4±1.0	2.8±.3	2.0±.2	504±57	389±47
2.0 to 2.5.....	24	1.27±.06	.93±.05	14.3±2.2	14.0±2.4	3.6±.6	2.8±.4	741±92	403±78
2.6 to 3.2.....	19	1.72±.05	1.23±.08	37.5±5.6	36.7±5.4	4.5±.6	3.1±.8	758±106	328±94
3.3 to 6.4.....	21	2.30±.11	2.22±.12	68.6±7.7	68.2±7.2	4.6±1.0	4.5±.8	521±80	462±74

DISCUSSION

The data presented in this study show that follicular development in the calf ovary is well under way a few days after birth and in general is augmented with age. This suggests that there is an increasing gonad-stimulating activity on the part of the anterior pituitary long before the time of puberty. The significant correlation between total volume of larger follicles and diameter of the uterine horn further suggests that follicular development in the ovary is not merely a morphological phenomenon but that the follicles in these sexually immature calves are to a degree physiologically active and producing an effect in uterine development. The trend of ovarian development demonstrated by this study appears to substantiate the idea that the morphological state and physiological activity of the ovary which are attendant upon puberty are the product of a gradual development, and are not effected briefly before reproductive maturity is attained.

On the basis of the mere presence of vesicular follicles, it would seem likely that almost all dairy calves of veal age should react experimentally to gonad-stimulating hormones. The minimum size of the vesicular follicle which is necessary to give the reaction is not determined by this study; but, considering the comparatively large size of the follicles in most calves, probably the majority of them would react to pituitary extracts.

The normal variability in calf ovaries indicates the necessity of determining the condition in each animal before experimental treatment. Especially is this true if the change to be produced is expected to be small. The normal values of the different characters reported in this study may be helpful in determining the degree of change produced experimentally in terms of normal change occurring with increasing body and ovarian weights.

The presence of follicles of ovulation or immediately preovulation size which do not rupture emphasizes the need for determination of the estrin content of follicles of different sizes and also the conditions in the mucosa and musculature of the genital tracts which are associated with them. The increased diameter of the uterine horn may indicate the functional activity of the ovary with regard to increasing secretion of estrin, but at what stage of its development the follicle begins to produce a physiological effect on the genital tract is unknown.

It seems unlikely that the large follicles which are present in the young calf persist in the ovary until puberty. While in general the number and size of large follicles appear to increase with the age of the animal, much variation occurred throughout the series and an appreciable number of calves in the younger group showed greater follicular development than some calves in the older group. It could not be determined in this study whether there were periodic waves of follicular growth in the ovaries as a whole, but it would seem that individual follicles develop and regress somewhat independently of other follicles in the same animal. The consistent occurrence of blood follicles in all groups of calves suggests that a rather continuous follicular degeneration takes place during the first few months of post-natal life. The number of enlarging follicles present at any one time, however, tends to increase as the animal grows older.

SUMMARY

Ovarian and follicular development and their relation to body weight and uterine size have been studied in 273 dairy calves, ranging from a few days to approximately 14 weeks of age as estimated from dressed weight. Vesicular follicles were present in practically all animals. Ovarian weight and degree of development of larger follicles were significantly correlated with both body weight and uterine diameter. Although a high degree of association existed between the right and left ovary with respect to weight and number of follicles, there were significant differences between the mean weight and the total follicular volume of follicles 4-13 mm in diameter in the two ovaries, the right having the greater values. Approximately one-half of the calves (irrespective of weight) had blood follicles present in their ovaries, suggesting continuous follicular degeneration during early postnatal life.

EFFECT OF FERTILIZERS, SOIL TYPE, AND CERTAIN CLIMATIC FACTORS ON THE YIELD AND COMPOSITION OF OATS AND VETCH¹

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INTRODUCTION

Climatic conditions as well as soil type and fertilizers may be expected to influence the yield and composition of crops. Although much work has been done to determine the effect of fertilizers and soil types on the yield and composition of crops, very little information is available on the influence of climatic factors when the same soil or culture solution is used under different climatic conditions the same year. The conclusions of Dickson (6)³ that oats grown at Madison, Wis., contained a higher percentage of calcium and phosphorus than oats grown at Pullman, Wash., under the same cultural conditions were not based on work done the same year but in different years. Variations in climatic conditions in the same locality from year to year may also be expected to result in differences in composition of crops. Likewise the report of Delwiche and Totttingham (5) to the effect that the nitrogen content of corn, barley, and clover was only slightly influenced by climatic conditions is open to question as these crops were grown on two different soil types, which by their very nature may cause differences in the composition of crops. It is apparent, therefore, that while the effect of fertilizers on crop yield is generally conceded, any differences in composition that may result from applications of fertilizers, differences in soil type, and variations in climate can be determined with certainty only when the same soils receiving identical treatments are subjected to different climatic environments the same year.

In an attempt to obtain some light on this subject a series of pail experiments with three widely different soil types was started in 1931. The experiments conducted at Pullman were duplicated in southwestern Washington in the area from which these three soils were obtained. In this manner the same soil receiving identical cultural treatments was subjected to two different climatic environments, the humid climate of southwestern Washington and the semiarid climate of eastern Washington.

The climate of southwestern Washington is of the marine type, having a narrow annual temperature range, an annual mean temperature higher than the average for the latitude, and heavy precipitation with a maximum in the winter months. Associated with the high rainfall is a high relative humidity and a large percentage of cloudy or partly cloudy days which may be expected to result in a

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³ Reference is made by number (italic) to Literature Cited, p. 974

low-light intensity. The elevation of this area is relatively low; that of the city of Centralia, which is centrally located in the experimental area, is only 182 feet above sea level. The climate of eastern Washington is essentially continental in character, having a wide annual temperature range. The precipitation is light and occurs largely in the winter period. The relative humidity is low, the number of clear days large, and the light intensity high. The elevation of this area ranges from 1,000 to 2,700 feet, that of the city of Pullman being 2,550 feet.

EXPERIMENTAL PROCEDURE

The three soil types selected for this experiment were (1) Felida silt loam from Clarke County, a soil derived from ancient alluvial or marine sediments; (2) Olympic loam from Cowlitz County, a soil derived from residual material; and (3) Chehalis clay loam from Lewis County, a soil derived from alluvial material. The normal annual precipitation in these three areas is approximately 37, 63, and 45 inches, respectively.

The samples of soil were obtained from near the check plots of experimental fields previously established by the Washington Agricultural Experiment Station. Soil taken from the upper 8 inches was thoroughly mixed, half of the amount sent to Pullman, and the other half retained by the cooperators in southwestern Washington. Each sample was sufficient to fill, in each of the two locations, 12 galvanized pails of 12-quart capacity to a depth of 8 inches. The pails were painted with asphaltum paint and provided with drains. Six pails were planted to oats (*Avena sativa* L.) and six to vetch (*Vicia sativa* L.). Each of the two sets of experiments received the following treatments: Check, N, NP, NPCa, NPKCa, and NCa.

Since the primary interest was in the mineral content of the crops, nitrogen was added to all pails except the check so as to eliminate the possibility of its being a limiting factor in plant growth. Chemically pure chemicals were used to avoid so far as possible the complicating effect of certain elements that might otherwise have been introduced. The diameter of the pails, 4 inches from the bottom, served as a means of calculating the area on which to base the rate of fertilizer application. The chemicals applied and their equivalents in fertilizers calculated on the acre basis are given in table 1.

TABLE 1.—Chemicals applied to pails of soil and their equivalents in fertilizers calculated on the acre basis

Treatment	Materials added	Fertilizer equivalent ¹	Approximate rate per acre
Check:			<i>Pounds</i>
N.....	NaNO ₃	Sodium nitrate.....	300
NP.....	NaNO ₃	do.....	300
	(NaH ₂ PO ₄) ₂ ·4H ₂ O.....	Superphosphate.....	600
	Ca(NO ₃) ₂ ·4H ₂ O.....	Sodium nitrate.....	300
NPCa.....	CaH ₂ (PO ₄) ₂ ·H ₂ O.....	Superphosphate.....	1,200
	CaCO ₃	Lime.....	4,000
	NaNO ₃	Sodium nitrate.....	300
NPKCa.....	KNO ₃	Muriate of potash.....	270
	CaH ₂ (PO ₄) ₂ ·H ₂ O.....	Superphosphate.....	1,200
	CaCO ₃	Lime.....	4,000
NCa.....	Ca(NO ₃) ₂ ·4H ₂ O.....	Sodium nitrate.....	300
	CaCO ₃	Lime.....	4,000

¹ Sodium nitrate, 15.5 percent N; superphosphate, 17 percent P₂O₅; muriate of potash, 48 percent K₂O; lime, 100 percent CaCO₃.

The chemicals were thoroughly mixed with the soil and sufficient water was added to bring the moisture content to normal field capacity as expressed by Shaw (14). The pails were covered and allowed to stand 3 or 4 days before seeding. Following the harvest, the soils were allowed to dry and were stored under cover during the winter. The second year chemicals were again mixed with the soil as in the previous year, water was added, and the moist soils were allowed to stand a week before seeding.

The three soils studied in southwest Washington, referred to as "the coast", were located at Chehalis in 1931, as were the Chehalis and the Felida soils in 1932. The Olympic soil was located at Woodland in 1932. The work in these localities was done by two cooperators and was conducted in the same manner as at Pullman insofar as it was possible to do this under cooperative arrangement.

Swedish Select oats and common vetch, grown on the college farm at Pullman, were used as seed. The vetch seed was inoculated with a culture of the proper legume bacteria. In both localities the oats and vetch were planted the same day at the rate of 20 seeds per pail. After the seedlings were well established they were thinned to 10 plants.

Each pail was weighed at the outset and the plants were watered frequently (every day during the latter part of the growing season). The pails were made up to weight once a week if necessary by the addition of water. Condensed steam served as a source of water at Pullman. Since neither condensed steam nor distilled water was available at Chehalis and Woodland, the regular city water was used. Although the water in these areas is known to have an extremely low mineral content, a chemical analysis revealed that it contained 7.5 parts per million of calcium, the chief element in water that might influence the results.

Both oats and vetch were harvested simultaneously, for any one soil, at a time when most of the oat plants were in the soft dough stage. The crops on the coast matured a week to 10 days later than at Pullman. After harvest, the crop samples were oven-dried at 65° C., weighed, and ground for chemical analysis.

Nitrogen was determined by the official Kjeldahl method (2).

For the determination of the mineral elements the oven-dried plant material was ashed in a muffle furnace at a dull red heat. The ash was taken up with 1:4 hydrochloric acid, diluted with hot water, and filtered into volumetric flasks. Phosphorus was determined by the official method (2) with slight modifications, calcium by Chapman's method (4), and potassium by the sodium cobaltinitrite method of Volk and Truog (15). In all cases the composition is reported in terms of percentages based on oven-dry weight of the plant material.

CLIMATIC CONDITIONS

The available weather data for the coast area are not as complete as those for Pullman, but the United States Weather Bureau data for Centralia, which is 4 miles distant from Chehalis and located in the same river valley, are considered fairly representative and are the best that are available for this purpose. The weather data for the growing seasons of 1931 and 1932 for both localities are given in table 2.

It is noted that the mean minimum temperature for the months of May, June, July, and August in both 1931 and 1932 was lower at Centralia than at Pullman. The mean maximum temperature at Centralia was higher during May and June and lower during July and August than at Pullman. Thus it is evident that the daily temperature differences were greater on the coast, especially during May and June.

In 1932 Pullman experienced a much greater number of clear days than Centralia and less than one-third as many cloudy days. In 1931 the difference in the character of the day for the two localities, although appreciable, was not as great.

Pullman received only 1.81 and 1.84 inches of precipitation during the growing seasons of 1931 and 1932 respectively, as compared to 5.13 and 3.35 inches for Centralia. Since the plants were watered regularly, they were not dependent on the rain for their moisture; but in all probability the difference in the precipitation and number of clear days had a direct effect on plant transpiration and the relative humidity of the air.

TABLE 2.—Climatic data for Pullman and Centralia, Wash., from May 10 to August 10, 1931 and 1932

PULLMAN								
Year and month	Mean temperature		Character of day			Days when precipitation was —		Precipitation
	Maxi- mum	Mini- mum	Clear	Partly cloudy	Cloudy	Less than 0.2 inch ¹	Over 0.2 inch	
	° F.	° F.	Number	Number	Number	Number	Number	Inches
1931:								
May.....	69.4	46.5	10	8	3	1	1	0.44
June.....	70.2	50.5	12	8	10	5	3	1.37
July.....	83.4	54.0	20	9	2	0	0	.00
August.....	82.0	54.4	10	0	0	0	0	.00
1932:								
May.....	63.9	43.9	12	5	4	5	2	1.29
June.....	74.8	51.3	20	6	4	0	1	.24
July.....	78.8	53.4	21	9	1	3	1	.31
August.....	83.7	59.1	8	2	0	1	0	.00
CENTRALIA								
1931:								
May.....	74.3	43.2	10	6	5	4	2	0.86
June.....	71.6	47.8	7	8	15	11	9	4.18
July.....	81.1	51.3	24	5	2	2	0	.08
August.....	81.3	50.4	7	3	0	1	0	.01
1932:								
May.....	66.6	42.5	8	5	8	9	1	.58
June.....	76.0	47.5	18	6	6	5	1	.37
July.....	72.6	49.7	12	5	14	8	3	2.16
August.....	79.6	52.1	7	0	3	4	0	.24

¹ Includes days designated as trace.

Because of the heavy rainfall at Centralia in June of 1931, the total exceeding 4 inches, water stood in the pails for a day or two after heavy rains and temporary waterlogging resulted. This may have had a slight effect upon the development of the crops.

CROP YIELDS

EFFECT OF FERTILIZER TREATMENT AND SOIL TYPE

It is often observed that the application of the same fertilizers will not always give the same crop response under apparently similar conditions, perhaps because environmental factors other than fertilizers exert some influence.

The individual crop yields as a result of different fertilizer treatments, soil type, and climatic conditions showed extreme variation. Those of oats ranged from 10.0 to 77.5 g and those of vetch from 1.5 to 46.4 g per pail. Considering the average yields of the 2 years for each of the 2 crops as influenced by fertilizer additions alone, and disregarding soil type and climate, as shown in figure 1, it is evident that the 2 crops responded very differently to fertilizer treatments. The addition of nitrogen alone resulted in a marked increase in yield of oats but depressed the yield of vetch. When phosphate was added in addition to nitrogen, both crops responded about equally in yield, as compared to the nitrogen treatment. There is no clear-cut evidence that the doubling of the amount of phosphate fertilizer contributed materially to crop yields since the apparent increase in yield of vetch resulting from the NPCa treatment, which contained twice as much phosphate as the NP treatment, may have been caused by the addition of lime.

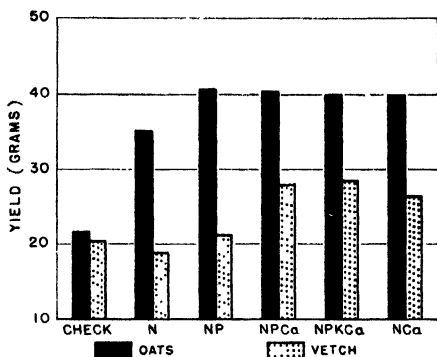


FIGURE 1.—Effect of fertilizers on yields of oats and vetch (average of all experiments).

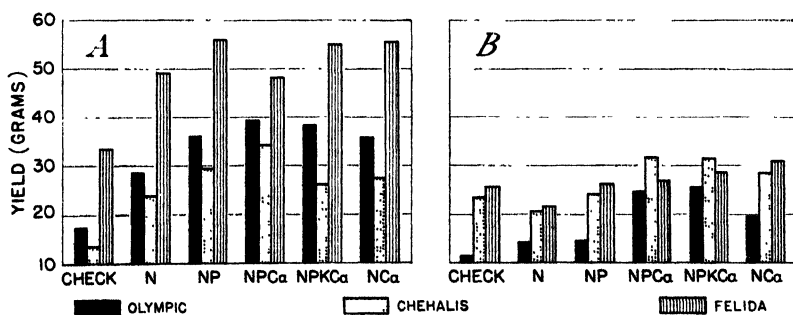


FIGURE 2.—Effect of soil type on yields of oats (A) and vetch (B) (average of 2 years and both locations).

When lime was used in combination with other fertilizer elements, the oat yields were not materially affected, but the vetch yields were materially increased.

Potassium fertilizer in combination with the other fertilizers had very little effect on yield.

When soil types alone are compared, as in figure 2, it is evident that the same general effects are indicated but with greater or less

variation. Of the three soils studied, Felida demonstrated the greatest natural productivity and also produced the greatest yields as a result of fertilizer additions, except that the NPCa and the NPKCa treatments when used for vetch gave slightly better results on the Chehalis soil.

The Olympic soil produced the lowest average yield of vetch. Vetch grown on this soil at Pullman the second year gave exceedingly low yields in the absence of lime—an average of 3.5 g—and high yields in the presence of lime—an average of 38.3 g. Since this condition was observed only at Pullman, it was decided to repeat the experiment with vetch on this soil the third year. The same relative results were obtained. This observed difference in response between the two locations may be partially due to the small amount of calcium present in the water used on the coast.

EFFECT OF CLIMATE

Although certain tendencies in the yield of oats and vetch appear to be characteristic effects of fertilizer treatments and soil types, climatic variations may also be expected to modify these results.

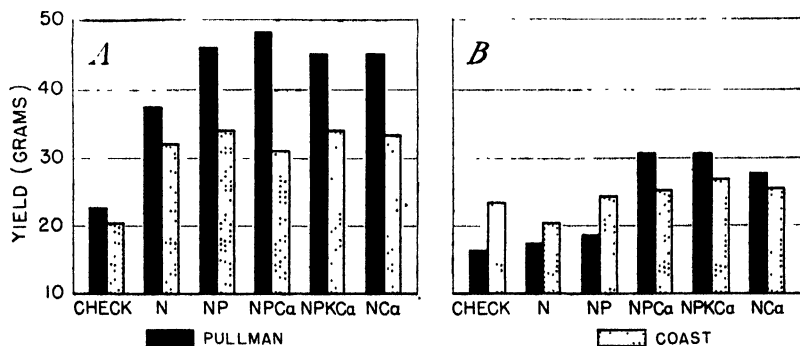


FIGURE 3 - Effect of local climate on yields of oats (A) and vetch (B) (average for all soils for 2 years)

The average yield for all soils for 2 years for each crop in each location is shown in figure 3. It is observed that oats yielded more at Pullman than on the coast, especially in the soils treated with P, K, and Ca in various combinations. Vetch in the presence of lime also yielded more at Pullman than on the coast. However, in the absence of lime higher yields of vetch were obtained on the coast.

The climatic effect is not necessarily limited to different localities but may exert its influence in the same locality in different years, as has been pointed out by Caldwell (3) and as can be seen from an analysis of the data presented by Delwiche and Tottingham (5), Dickson (6), and Frankena (10). Similar results are indicated in figure 4, where the average yields of oats and vetch for the three soil types are illustrated for 1931 and 1932.

The yield of oats was greater at Pullman than on the coast in 1931 but the reverse was true the following year except for the NPCa treatment. The difference in the yield of vetch between the two localities was not as great as for oats the first year, although in general the better yields were obtained at Pullman. In 1932, vetch in the

absence of lime gave considerably greater yields on the coast but when lime was present, as already explained, the yields at Pullman were greater. This difference in response the second year was probably due to an actual shortage of calcium as a nutrient, and as a result of a greater total removal of calcium at Pullman this deficiency appeared there first.

The effectiveness of any particular fertilizer, as indicated by yields, may vary with the different seasons, as is shown in figure 4. In 1931 in both locations the addition of nitrogen to vetch increased the yield, whereas in 1932 the yield was reduced when nitrogen was added. This lack of response the second year may have been due to better inoculation.

When phosphate was supplied in addition to nitrogen, the yield of vetch grown on the coast was reduced in 1931 and increased in 1932, while at Pullman the reverse was true.

At Pullman the addition of lime reduced the oat yields in 1931 and increased them in 1932; it was only slightly beneficial for vetch in 1931 but greatly increased the yield in 1932. On the coast the addition of lime had very little influence on the yield of either oats or

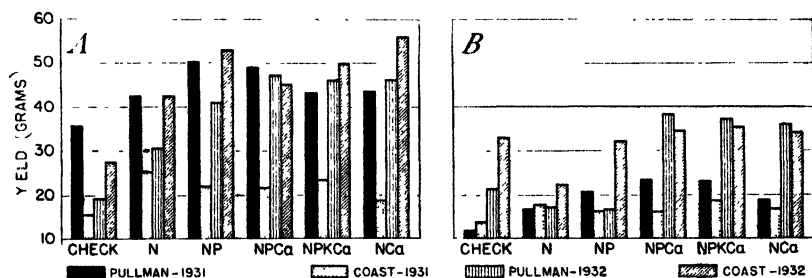


FIGURE 4.—Effect of local climate in different years on the yield of oats (A) and vetch (B) (average for all soils), 1931 and 1932.

vetch in 1931 or 1932. The small amount of lime supplied by the water used may partially account for the lack of response.

A careful analysis of the data on yields reveals certain outstanding facts. The results clearly indicate that in evaluating the effect of fertilizers on crop yields, the kind of crop, the soil type, and the climatic environment must be considered as well as the kind and amount of fertilizers applied. Although suitable quantities of the proper combination of fertilizers resulted in a generous crop response in all cases, the average increase in yield for the 2 seasons on the 3 soils irrespective of location was approximately 87 percent for oats and only about 42 percent for vetch. As for the influence brought about by soil type disregarding climatic environment, the extremes in the average increase in yield for oats for the 2 seasons varied from 155 percent for the Chehalis soil to 67 percent for the Felida soil, and for vetch from 126 percent for the Olympic soil to 21 percent for the Felida soil.

It is a common experience in field experiments to find that repeated applications of the same fertilizers to the same soil do not give the same results from year to year. Variations in available moisture and especially a deficiency of moisture are generally accepted as the major

responsible factors. Since these factors were entirely eliminated in these experiments by providing an adequate supply of available moisture for the crops at all times, other climatic factors undoubtedly had an important bearing on the crop growth. Arthur (1) pointed out that light intensity and light quality as well as other climatic factors and soil conditions affect plant growth. When the yields of the 3 soils for the 2 seasons are averaged, the results show that the most favorable fertilizer treatment resulted in a 111 percent increase in the yield of oats at Pullman and only an 89 percent increase on the coast. The yield of vetch, when calculated on the same basis, returned a 67 percent increase at Pullman and only a 13 percent increase on the coast.

CHEMICAL COMPOSITION

EFFECT OF FERTILIZER TREATMENT AND SOIL TYPE

The effect of fertilizers on the composition of crops is somewhat of a controversial matter, perhaps because environmental factors other than fertilizers exert their influence. Gericke (11) suggests that some of the unexplained discrepancies that commonly are encountered in comparative studies on plant salt requirements and the application of fertilizer to agricultural soils may be related to climatic influences.

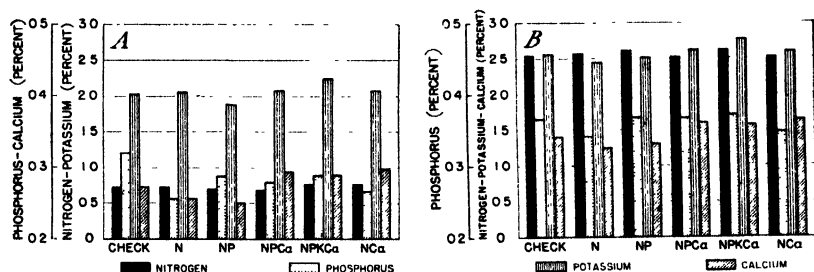


FIGURE 5.—Effect of fertilizers on the chemical composition of oats (A) and vetch (B) (average for all soils for 2 years and both locations).

Considering the effect of fertilizers from the standpoint of crops alone, and disregarding soil type and climate as is indicated in figure 5, the average composition of oats and vetch grown during two different seasons was most affected by the additions of phosphate fertilizers and lime. When a phosphate fertilizer was added to the soil, the percentage of phosphorus in both oats and vetch was increased above that in oats and vetch which received fertilizer other than phosphate but not above that in the unfertilized plants. The addition of lime to the soils caused an increased percentage of calcium in both oats and vetch above that of plants grown on soils which received no lime. The presence of lime also slightly increased the potassium content of both oats and vetch. The addition of nitrogen alone definitely reduced the percentage of phosphorus and calcium in both oats and vetch. This tendency seemed to be apparent in regard to the phosphorus content of oats even when nitrogen was used in combination with the other fertilizers. This may be partially accounted for by increased yields resulting from the nitrogen fertilizer, since the oats were considerably more affected in this respect than the vetch.

When soil types alone are considered, however, it is evident that although the same general effect is indicated the different soils show considerable variation, especially in the phosphorus and calcium content of both crops. This is shown in figure 6. Oats and vetch grown on Olympic soil, in general had a lower phosphorus and calcium content than those grown on the other soils. The application of phosphate to the Olympic soil did not generally increase the phosphorus content of these crops, but when applied to the Felida and

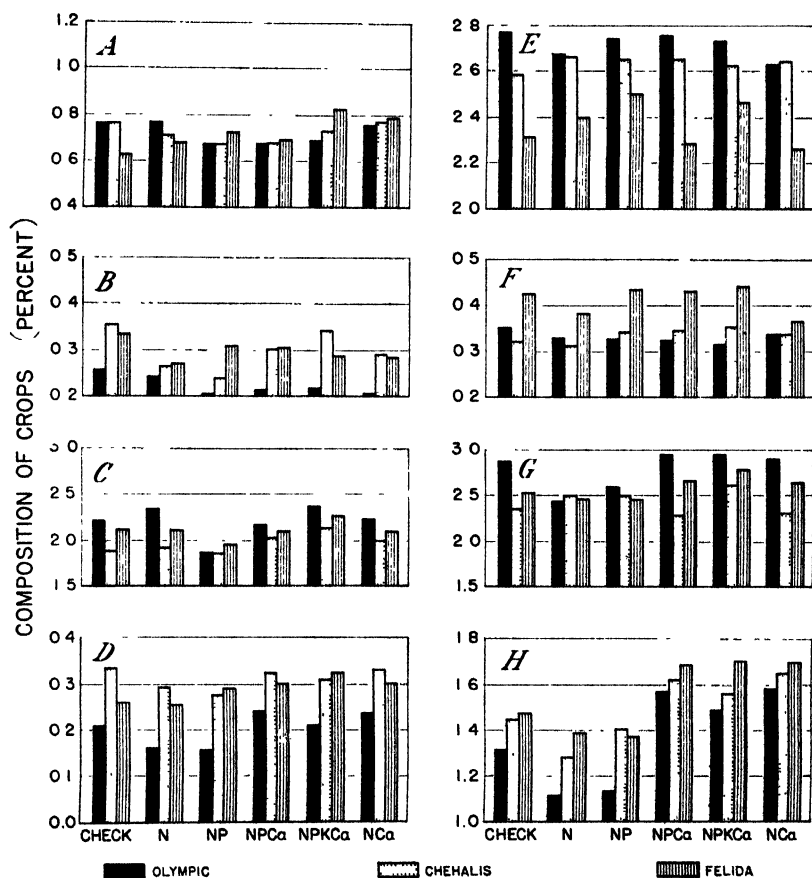


FIGURE 6.—Effect of soil type on the chemical composition of oats and vetch (average for 2 years and both locations): A to D, Oats; E to H, vetch; A and E, nitrogen content of crop; B and F, phosphorus content of crop; C and G, potassium content of crop; D and H, calcium content of crop.

Chehalis soils, it resulted in a uniform rise of the phosphorus content of the vetch but not of the oats. The addition of lime markedly increased the calcium content of oats on the Olympic soil and only slightly on the other soils. The influence of soil type was less apparent in regard to the calcium content of vetch when lime was used, but again the greatest effect was obtained on the Olympic soil. Fonder (7, 8, 9) also found a considerable variation in the magnesium as well as in the calcium content of alfalfa, beans, and peas when grown on

different soil types under the same general climatic conditions in the same locality the same year. The general depressing effect of nitrogen fertilizer on the phosphorus content of the crops, although greater on the Olympic soil, was never completely overcome on either the Chehalis or the Felida soil by any of the fertilizer combinations regardless of the relatively large amounts of phosphate fertilizers used in some of the treatments.

The nitrogen and potassium content of the crops was much less affected by soil type. The results for nitrogen are in accord with those obtained by Delwiche and Tottingham (5) and Le Clerc (12), who found that the percentage of nitrogen in the crops they studied was influenced only slightly by soil type even though great variation in types existed.

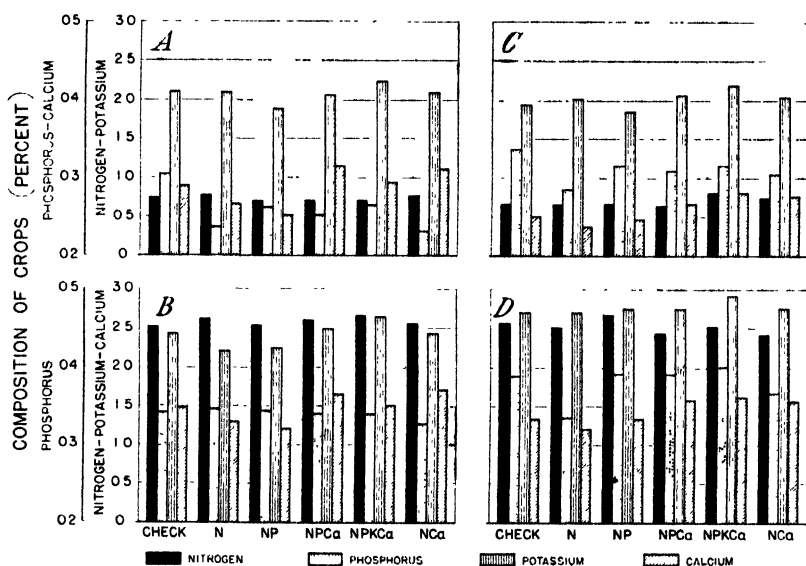


FIGURE 7.--Effect of local climate at Pullman (A, B) or on the coast (C, D) on the chemical composition of oats (A, C) and vetch (B, D) (average for all the soils for 2 years).

The percentage of a specific element in a plant may be somewhat dependent on the interaction of the soil with the specific element added as a fertilizer. For example, when phosphate in combination with nitrogen was added to the Olympic soil, it resulted in a decrease in the phosphorus percentage of both oats and vetch below that of nitrogen alone but when added to Felida an increase in phosphorus content was observed.

EFFECT OF LOCAL CLIMATE

Although certain definite tendencies in the composition of oats and vetch appear to be characteristic effects of fertilizer treatments and soil types, these are undoubtedly modified by climatic influences. This was very strikingly shown when the average composition of the two crops was considered, as indicated in figure 7. It was found that the percentage of phosphorus was higher and the percentage of calcium

was lower on the coast than at Pullman. This phosphorus-calcium-climate relationship has been observed also in unpublished work done in this laboratory when the composition of alfalfa grown on the coast was compared with that of alfalfa grown in the irrigated valleys of the central part of the State. This cannot be entirely attributed to annual climatic changes, as the same general comparison was observed in 1931 and in 1932.

The data in table 2 show that the total number of clear days was greater and the precipitation lower at Pullman than on the coast for both years. This fact, which corresponds with the results of Mac-Millian's work (13) in which he concluded that localities with higher elevation and lower relative humidities receive a greater total amount of ultraviolet rays, indicates that the amount of ultraviolet light received at Pullman should be greater than that received on the coast for both years. Since Wynd and Fuller (16) found that tomato and cucumber plants subjected to ultraviolet radiation contained a higher percentage of calcium and a lower percentage of phosphorus than unirradiated plants, it seems that the amount of ultraviolet radiation may be one of the important factors that determined the observed phosphorus-calcium relation between the two different localities.

Applications of lime were more effective in increasing the calcium content of both oats and vetch at Pullman than on the coast. The calcium content of the water used on the coast did not have any appreciable effect on the observed results if the chemical composition of the oats and vetch grown is used as a criterion, for in all cases except the NP and NPKCa treatments of the latter crop the calcium content was lower on the coast.

There was very little difference in the nitrogen and potassium content of the oats grown in the two localities, while for vetch the potassium content was higher on the coast and the nitrogen content higher at Pullman.

The climatic effect was not limited to different locations but showed its influence in the same localities in different years. This was also shown in the work of Caldwell (3), Frankena (10), and Dickson (6). The results are illustrated in figure 8 in which the composition of the same crop grown in the same locality is graphed separately for each year.

The percentage of nitrogen in vetch was higher in 1932 than in 1931 for both localities, while in oats the nitrogen content was higher at Pullman in 1931 than in 1932 and on the coast no consistent results were observed. The phosphorus and potassium content of both oats and vetch, on the coast and at Pullman alike, was more or less greater in 1931 than in 1932, but the calcium content was not uniformly affected by seasonal climatic variations in either locality.

The existing differences in the mean daily temperature, in condition of the day, and in precipitation in the same locality in different years did not seem to exert any appreciable effect on composition. It is possible that other climatic factors may be the dominant ones as far as climate is concerned when composition changes are produced, or the cause may be a balance between all climatic factors.

In recent years considerable significance has been attached to the phosphorus and calcium content of feeds for livestock. It has been found that livestock fed on pasturage and hay containing these

elements in amounts below a certain critical figure suffer from nutritional diseases. The many controversial data presented in the literature on the effect of fertilizers on the composition of crops cast doubt upon the practicability of improving the mineral content of forage crops by means of fertilizer applications to the soil. However, in the large majority of these cases no consideration has been given to the influence of soil type and climatic factors, both of which have clearly demonstrated their influence in the work here reported. Moreover, in many of the instances reported in the literature the phosphorus and calcium content of the crops grown without fertilizers

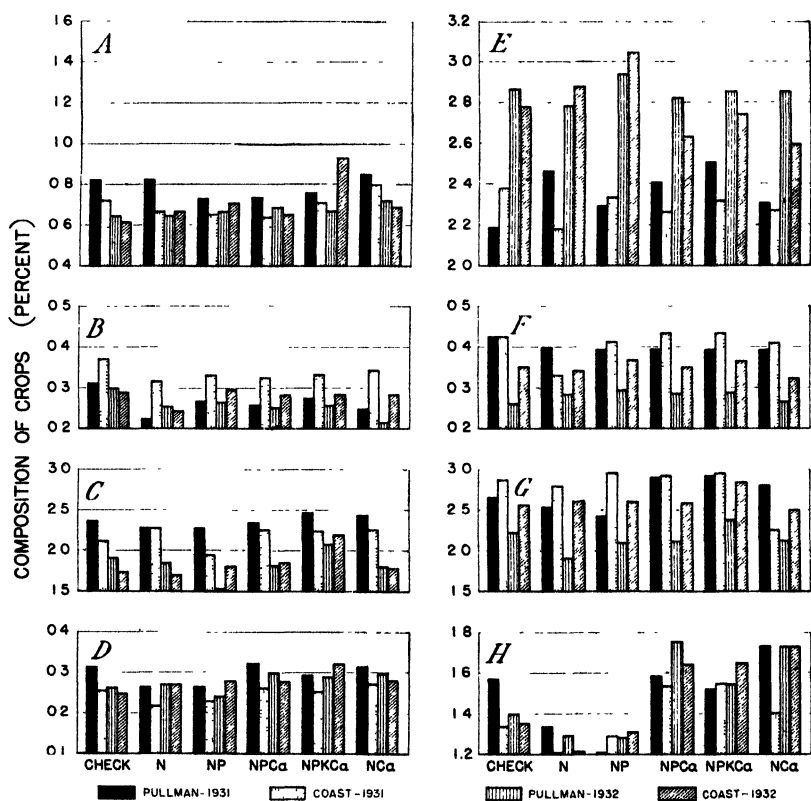


FIGURE 8.—Effect of the climate of different years on the chemical composition of oats and vetch (average of all soils), 1931 and 1932: A to D, Oats; E to H, vetch; A and E, nitrogen content of crop; B and F, phosphorus content of crop; C and G, potassium content of crop; D and H, calcium content of crop.

was not at the critical point, and consequently there was no urgent need for improvement of quality. The oats and vetch grown on the three soils employed here are typical examples. Nevertheless, applications of phosphates and lime to these soils counteracted the depressing effect of nitrogen on the phosphorus and calcium content of the crops, and although this beneficial influence was modified in varying degrees by soil type and climatic factors, it was general in all cases. If corresponding results can be obtained on soils that are so deficient in available phosphorus and calcium as to result in crops

with an abnormally low content of these elements, it seems that it should be possible to secure improved quality of crops as well as profitable returns when proper amounts of suitable fertilizers are applied to the soil.

SUMMARY AND CONCLUSIONS

Three of the more important soil types of southwestern Washington were selected for a series of pail experiments to study the effect of fertilizers, soil type, and local climate on the yield and chemical composition of oats and vetch. Duplicate sets of the same soil were used, one in the coastal climate of southwestern Washington in the locality from which the soil was originally obtained and the other in the semiarid climate at Pullman. The results indicate that fertilizers, soil type, and local climate may have an important effect upon the yield and chemical composition of crops.

Fertilizer additions materially changed the yield of oats and vetch, the two crops responding differently to applications of nitrogen and lime and similarly to applications of phosphate and potassium. The chemical composition of the crops was likewise influenced by the use of fertilizers. Additions of nitrogen depressed the phosphorus and calcium content of both crops while additions of phosphate and lime increased the phosphorus and calcium content of the plants above that of the plants which received nitrogen fertilizers alone but not above that of the unfertilized plants.

The inherent soil characteristics, reflected in soil type, had a marked influence on the yield and chemical composition of the two crops. The same plant species showed a different relative response to the same fertilizers when grown on different types of soil. The percentage of calcium and phosphorus in the crops was especially affected by soil type, while the percentage of nitrogen and potassium was only slightly affected.

Climatic differences, excluding moisture, between the two localities also influenced the yield and chemical composition of oats and vetch. In general, the higher yields were obtained at Pullman. The same crops grown on the same soil under the same cultural conditions contained, on an average, a higher percentage of phosphorus and a lower percentage of calcium when grown in the coastal climate of southwestern Washington than they did when grown in the semiarid climate of Pullman. The effect of the addition of lime on the calcium content of both crops was more pronounced at Pullman than in southwestern Washington. The difference in the character of the light in the two localities probably resulted in a difference in the amount of ultra violet rays received by the plants, and this seemed to be related to the observed results in composition. It is evident that the dominant climatic factors involved are those other than temperature and precipitation.

The yield and chemical composition of the same crop on the same soil under the same cultural conditions may vary from year to year as a result of local climatic variations.

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PEAR ROOT CONCENTRATION IN RELATION TO SOIL-MOISTURE EXTRACTION IN HEAVY CLAY SOIL¹

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INTRODUCTION

In the study of pear irrigation in the Rogue River Valley of Oregon it became important to determine those soil zones from which moisture was extracted most rapidly and also to learn whether such zones of greatest moisture loss corresponded to zones of greatest root concentration. The work carried on during 1931 and 1932 in heavy clay soil shows a clear-cut relation between observed root concentration and rate of soil-moisture extraction and provides some definite information as to the actual distribution and concentration of pear roots in this heavy clay soil.

SOIL-MOISTURE EXTRACTION BY ROOTS

METHOD OF MEASURING

The rate of soil-moisture extraction by the tree roots was measured by determining the decrease in soil-moisture content at different depths and at different distances from the trunk, during specific periods when no moisture was added to the soil. Soil samples were taken with the improved King tube in 1-foot increments from the under side of the mulch to bedrock, the depth of sampling never exceeding 8 feet. In the case of trees X-40 and MM-33, with no cultivation since the previous fall, there was no mulch, so sampling started at the surface. Standard methods were used in determining the moisture content of the samples. Obviously erroneous determinations were discarded.

TREES USED IN 1931

In the Fitch orchard one 24-year-old Bartlett tree (French roots), in a block planted 24 feet on the square, was used. This tree had a trunk circumference of 48.5 cm and a top volume of about 270 cubic feet. The soil is Meyer silty clay loam, 5 feet deep to a sandstone bedrock. The last irrigation for the season, on June 24, brought the soil moisture almost to the field capacity.

In the Klamath orchard one 26-year-old Anjou pear tree (French roots), in a block planted 26 feet on the square, was selected. This tree had a trunk circumference of 68.7 cm and an average limb spread of about 6.2 feet radially from the trunk. The soil is Meyer clay

¹ Received for publication Feb. 18, 1935; issued July 1935. Cooperative studies on irrigation of pears near Medford, Oreg., carried on by the U. S. Department of Agriculture and the Oregon Agricultural Experiment Station.

adobe, 4 feet deep to shale and marl bedrock. When this tree was irrigated for the last time, on July 16, the soil moisture was brought to 90 percent of the field capacity in the upper 4 feet.

Soil samples were taken along the four diagonals, the first sample 2 feet from the trunk and each succeeding sample 2 feet farther out, on the diagonal next in order, the outermost sample being 18 feet from the trunk. In addition, duplicates at 6 and at 12 feet from the trunk were taken on one side of each tree.

TREES USED IN 1932

Two large and vigorous Anjou pear trees (French roots) in the Medford experiment station orchard were used. Tree X-40 had a trunk circumference of 73 cm and about 39,000 leaves. Tree MM-33 had a trunk circumference of 96 cm and about 70,000 leaves. The limb tips of tree MM-33, in a block planted approximately 26 feet on the square, nearly met those of adjacent trees; but between the limb tips of tree X-40, in a block planted approximately 30 feet on the square, and those of adjacent trees there was considerable space.

To isolate the soil mass around these two trees from the effect of roots from adjacent trees and from water seepage, trenches 20 inches in width were dug during March around four sides of a rectangle surrounding each tree. For Anjou X-40, in deep soil, the trench was excavated to the water table, a depth of 9 feet; for Anjou MM-33, in shallow soil, the trench was dug to a depth of 4 feet, or about 6 inches into the crumbling shale bedrock. After the inner sides of each trench were lined with single-ply tar paper fastened to boards extending 4 inches above the ground surface, the seams in the paper were tarred and the trench was filled in. The soil surface was left uncovered and all weeds were removed. The upward or downward movement of moisture from the soil mass was unobstructed by artificial barriers. With good natural drainage and practically no summer rainfall, no drainage of the soil mass, as provided in the experiments of Oskamp (11),² was necessary. For tree X-40 the isolated soil mass was 30 feet square; for tree MM-33 it was 27.0 feet long and 25.5 feet wide.

One sampling location was at the base of the tree. On each of one pair of opposite diagonals samples were taken at 2, 6, 10, 14, and 18 feet, respectively; on each of the other pair samples were taken at 4, 8, 12, and 16 feet, respectively.

To ascertain soil-moisture variations unaffected by active tree roots, similar walls were put around two cylindrical cores of undisturbed soil, 36 inches in diameter, one core being adjacent to each of the trees. Soil-moisture samples from these cores free of active roots were taken at the same dates as those from the root-containing area.

IRRIGATION PLOTS USED IN 1932

During 1932 additional information upon the relative soil-moisture decreases at different positions around moderate-sized Anjou trees was available in the soil-moisture data from four experimental irrigation plots. In these plots, each containing 0.88 acre and from 14 to 17 experimental trees, soil samples were taken in 1-foot increments to bedrock lying at depths of 4 to 6 feet, at definite locations 4, 7.5,

² Reference is made by number (*italic*) to Literature Cited, p. 987.

10, 11.5, and 13 feet from the trunks. In each plot, these positions were quadruplicated. Thus for each plot there were at each sampling from 16 to 24 separate determinations (four series, each consisting of one sample for each foot in depth) for each distance from the tree, and 20 separate determinations for each depth to 4 feet. The periods of soil-moisture decrease during the summer, selected for study of water extraction by the roots, were not the same for all plots.

SOIL-MOISTURE EXTRACTION AT DIFFERENT DEPTHS

After the spring cover crop had been disked under, one or more periods during the summer when soil-moisture decrease was rapid and unaffected by rainfall or irrigation were selected for each tree or group of trees. During these periods soil moisture did not decrease to the wilting point³ at any depth. The average soil moisture content for each foot in depth, based on the average for all distances from the trunk, was calculated for the beginning and for the end of each selected period. The difference between these two averages gave the average soil-moisture decrease for any one depth during this period. Because the total amount of moisture extracted was not the same for all trees or plots, the amount of moisture extracted in each case was expressed as percentage of the sum of the moisture decreases for all depths. This percentage, considered the relative rate of moisture extraction for that depth, is then comparable with that for any other tree or plot.

For the Bartlett and Anjou trees studied in 1931, it was assumed that moisture loss by direct evaporation from the soil was too slight to be considered. The extent of such loss was measured in 1932 by the isolated cores of soil, containing no active roots. It was found that in the top foot of the core the moisture decreased for about 30 days, but in the lower depths moisture changes were negligible. The moisture decrease in the top foot of the core for tree X-40 was 0.07 percent per day, and for tree MM-33 it was 0.11 percent per day. Therefore, in calculating water extraction by the roots for trees X-40 and MM-33, this estimated loss was subtracted from the average soil-moisture decrease of the first foot only. Since evaporation loss from the four irrigation plots was not known, a correction of 0.092 percent per day (average of losses from trees MM-33 and X-40) was arbitrarily used for the first foot only in each plot.

The relative rates of extraction of moisture from each depth for Fitch orchard Bartlett, Klamath orchard Anjou tree X-40, tree MM-33, and the four irrigation plots are given in table 1.

The greater relative moisture extraction in the top foot of plot C (table 1) was perhaps partly due to the more frequent irrigation of this plot, which resulted in a continuously higher moisture content, probably with correspondingly greater surface evaporation loss, in the top foot of this plot than in the top foot of the other plots.

³ In this statement, "soil moisture" refers to average moisture content of sample of soil taken with King tube.

TABLE 1.—Relative rate of soil-moisture extraction at different depths and at different distances from the trunk for Fitch orchard Bartlett, Klamath orchard Anjou, tree X-40, tree MM-33, and the four irrigation plots

AT DIFFERENT DEPTHS¹

Depth or distance (feet)	Fitch orchard Bartlett	Klamath orchard Anjou	Tree X-40 ²	Tree MM-33	Irrigation plot				Average ³ for trees in soil 4 feet deep
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
0-1.....	39	35	430	434	432	435	445	433	34
1-2.....	25	27	35	28	27	28	23	23	28
2-3.....	18	20	23	23	22	25	13	14	22
3-4.....	9	18	12	15	19	12	11	13	16
4-5.....	9	(⁵)	-----	(⁵)	(⁵)	(⁵)	8	12	-----
5-6.....	(⁵)	(⁵)	-----	(⁵)	(⁵)	(⁵)	(⁵)	5	-----

AT DIFFERENT DISTANCES FROM TRUNK⁶

0.....	50	55	75	120	-----	-----	-----	75±10.7
2.....	80	80	100	135	-----	-----	-----	99±8.7
4.....	135	125	105	110	105	90	145	116±4.3
6.....	90	95	105	100	-----	-----	-----	98±2.2
7.5 or 8.....	120	110	130	100	100	130	95	112±3.2
10.....	85	80	85	90	95	95	95	100
11.5 or 12.....	80	110	105	100	100	105	65	91±4.4
13 or 14.....	80	75	75	100	100	80	100	90±3.3
16.....	80	100	75	105	-----	-----	-----	90±15.0
18.....	85	130	35	95	-----	-----	-----	80±13.2

¹ Extraction at each depth is expressed as percentage of total extraction for all depths

² Calculated for top 4 feet only, although soil was 9 feet deep.

³ Based on Klamath orchard Anjou, tree MM-33, and irrigation plots A and B.

⁴ Corrected for surface evaporation.

⁵ Rock occurred at this depth

⁶ Extraction at each distance is expressed as percentage of average of extraction at 4, 8, 10, 12, and 14 feet for individual trees, and at 4, 7.5, 10, 11.5, and 13 feet for irrigation plots.

For the trees in soil just 4 feet deep, the first foot showed the greatest average extraction (34 percent); the top 2 feet, 62 percent; and the top 3 feet, 84 percent. The 3- to 4-foot zone showed only 16 percent. Although some roots penetrated the underlying shale bedrock, the moisture extracted by such roots was probably very small in proportion to that extracted in the top 4 feet.

SOIL-MOISTURE EXTRACTION AT DIFFERENT DISTANCES FROM TRUNK

In the determination of the soil-moisture extraction at each distance from the trunk, the average for all depths was used. Since the sampling locations for the individual trees were not all at the same distance from the trunk as were the locations for the irrigation plots, a special method of presentation has been used to make all the data comparable. The sampling locations for the individual trees at 8, 12, and 14 feet from the trunk are considered comparable to the locations at 7.5, 11, and 13 feet, respectively, for the irrigation plots. The relative rate of moisture extraction at any location for the individual trees has been expressed as percentage of the average extractions of locations 4, 8, 10, 12, and 14 feet from the trunk; whereas the relative rate of moisture extraction for any location in the irrigation plots has been expressed as percentage of the average extractions of locations 4, 7.5, 10, 11.5, and 13 feet from the trunk. The data, thus expressed, are given in table 1.

Both the individual trees and the irrigation plots show considerable variation in relative rate of moisture extraction at any given distance

from the tree. For the individual trees the small number of soil samples at each location might explain the variability, but for the irrigation plots the variation is more than might be expected for the average of 4 to 7 periods of extraction for four series of sampling holes. Such variation does not permit any meaning to be attached to tree or plot differences. However, the average relative rate of extraction for each approximate distance shows that the extractions at 4, 6, and 7.5 or 8 feet from the tree were slightly but not always significantly greater than at the base of the trunk or at distances greater than 8 feet from the trunk. At distances between 10 and 18 feet from the trunk the average rates of extraction were about the same.

ROOT DISTRIBUTION

ROOT COUNTS

When the trenches around trees X-40 and MM-33 were dug, the inner trench wall on two sides of each of the trees was divided into foot squares with chalk lines. When all roots along the wall were exposed by picking away the soil with a small ice pick, the number of roots below 1 mm in diameter in each square was recorded. The average number of such roots per square foot for each depth is given in table 2. Since there was no surface mulch, depths were measured from the soil surface.

TABLE 2.—Average number of roots less than 1 mm in diameter for each soil depth for trees X-40 and MM-33

[Data expressed as number of roots in 1 square foot on the side of a trench and as percentage of total]

Depth (feet)	Tree X-40 roots		Tree MM-33 roots		Depth (feet)	Tree X-40 roots		Tree MM-33 roots	
	Number	Per- cent	Number	Per- cent		Number	Per- cent	Number	Per- cent
0-1.....	9.6±0.58	11	14.8±0.74	28	4-5.....	2.0±0.10	2		
1-2.....	18.0±.81	21	16.6±.68	32	5-6.....	12.4±1.02	14		
2-3.....	19.9±.83	23	13.4±.55	26	6-7.....	7.3±.72	8		
3-4.....	12.4±.73	14	7.3±.47	14	7-9.....	6.3±.00	7		

For tree X-40 the greatest number of roots below 1 mm in diameter occurred in the second- and third-foot depths, with the next greatest number in the first-, fourth-, and sixth-foot depths; between 4 and 5 feet below the surface a sandy clay stratum containing deposits of lime had very few roots. Below 6 feet the silty clay-loam subsoil contained pockets of dark-colored clay, in which most of the roots at that depth were found.

The number of roots in each of the upper 4 feet, expressed as percentage of the total roots in the top 4 feet, was for the 0- to 1-foot depth, 16 percent; for the 1- to 2-foot depth, 30 percent; for the 2- to 3-foot depth, 33 percent; and for the 3- to 4-foot depth, 21 percent. This distribution of roots does not correspond to the relative rate of moisture extraction observed along diagonals extending out from the trunk (table 1). This lack of agreement may have been caused by vertical movement of soil moisture or by the difference in the root distributions at the soil-sampling locations from those in the trench profile. It is also possible that the root distribution nearer the trunk would differ somewhat from that obtained in a trench 15 feet from the trunk.

For tree MM-33, the first and second foot each contained about 30 percent of all the roots observed to a depth of 4 feet; the third foot contained 26 percent; and the fourth foot contained 14 percent. This distribution agrees fairly closely with the rate of moisture extraction at each depth for this tree (table 1), which showed 34, 28, 23, and 15 percent, respectively, in each foot between the surface and a depth of 4 feet.

ROOT WEIGHTS FOR TREE MM-33

In October 1932 the distribution of small visible roots around tree MM-33 was determined in order to learn how closely the concentration of such roots at different positions corresponded to the observed relative rate of moisture extraction. The distribution of visible roots was determined by a method somewhat similar to that reported by Rogers and Vyvyan (16) and by Furr and Magness (6). A trench 24 inches wide was dug along each of the four diagonals from the trunk to a distance of 14 feet from the tree. To a depth of 6 inches below the surface, the soil was removed separately from zones 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 14 feet, respectively, from the trunk. All the roots in each zone were separated from the lumps of sticky soil by hand, air-dried for approximately half an hour, separated into three groups according to size, and weighed to 5 mg. When the first 6 inches of soil in one trench had been removed, the next 6 inches was taken out in a similar manner; subsequently, the second 12 inches, the third 12 inches, and, finally, the lowest 12 inches. These operations were repeated for all four trenches. The weight of roots under 2 mm in diameter per cubic foot of soil (termed "root concentration") for each zone is given in table 3.

In view of the variability of root concentration for different locations, the trend of root distribution can be more clearly seen in the figures for average concentration for each depth and for each distance from the trunk.

The distribution of roots to a depth of 4 feet is shown in the next to the last column of table 3, which gives the average root concentration at each depth. It is evident that the greatest concentration of small roots occurred between 6 and 12 inches below the surface. This depth had nearly twice the average concentration of the 12- to 24-inch depth. The 24- to 36-inch depth and the 36- to 48-inch depth had each a lower root concentration than the layer just above. The 0- to 6-inch depth, which shows about one-quarter of the concentration found in the 6- to 12-inch depth, actually had almost no roots in the top 4 inches, as most of the roots were confined to a layer between 4 and 6 inches deep. The average concentration for the entire 0- to 12-inch depth, however, was about the same as that for the 12- to 24-inch depth.

The relative concentrations of roots for each foot in depth, expressed as percentages of the sum of the concentrations in the top 4 feet, given in the last column of table 3, were 35, 33, 21, and 11, respectively, for the first, second, third, and fourth foot in depth. These figures for root concentration are directly comparable with figures expressing the relative moisture extraction for these depths, which are (table 1) 34, 28, 23, and 15. Statistical comparison shows a correlation $+0.95 \pm 0.04$, indicating that for each depth, average moisture extraction was directly proportional to the concentration of small visible roots.

TABLE 3.—*Concentration of visible pear roots less than 2 mm in diameter, for tree MM-33, in clay adobe soil 3½ feet deep to shale bedrock*

[Data given as grams of roots per cubic foot of soil]

Depth (inches)	Trench location	Roots in soil zone at indicated distance from trunk							Average root concentra- tion	Relative root concentra- tion ¹
		0-2 feet	2-4 feet	4-6 feet	6-8 feet	8-10 feet	10-12 feet	12-14 feet		
		<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Percent</i>
0-6	SW	1.6	4.1	4.4	4.0	2.7	3.0	1.4		
	SE	1.4	3.6	2.5	2.4	3.9	1.4	1.6		
	NW	1.5	.9	1.4	.8	2.3	2.1	2.7		
	NE	1.5	4.9	2.0	1.3	1.8	1.8	1.6		
	Average	1.5	3.4	2.6	2.1	2.7	2.1	1.8	2.3	
0-12	SW	10.7	10.0	8.8	8.5	7.8	6.0	4.7		
	SE	5.3	18.6	11.0	12.2	7.0	5.7	4.3		
	NW	5.2	9.6	10.9	10.1	7.9	10.6	5.1		
	NE	5.4	7.8	14.4	11.0	12.4	11.0	15.1		
	Average	6.7	11.5	11.3	10.5	8.8	8.3	7.3	9.2	
0-12	do	4.1	7.4	6.9	6.3	5.7	5.2	4.6	5.7	35
12-24	SW	6.0	5.0	5.7	5.0	5.2	6.5	7.6		
	SE	8.4	6.7	6.0	5.4	4.9	4.5	3.8		
	NW	4.4	8.4	8.3	5.7	2.9	2.8	5.1		
	NE		3.9	8.6	4.4	4.2	4.1	4.8		
	Average	6.3	6.0	7.2	5.1	4.3	4.5	5.3	5.5	33
24-36	SW		4.0	3.1	5.6	3.8	4.8	3.0		
	SE		3.8	4.3	5.2	3.9	4.1	3.8		
	NW	2.2	3.1	1.8	3.1	2.0	3.1	2.3		
	NE		3.8	2.6	3.4	2.8	4.2	3.3		
	Average	2.2	3.7	3.0	4.3	3.1	4.1	3.1	3.4	21
36-48	SW		.9	2.3	2.3	2.1	1.8	.9		
	SE		2.1	2.2	2.1	2.7	2.0	2.1		
	NW	1.5	3.7	2.1	.5	.5	.5	1.1		
	NE		1.7	1.6	2.5	1.1	1.5	2.6		
	Average	1.5	2.1	2.1	1.9	1.6	1.5	1.7	1.8	11
0-48	do	3.5	4.8	4.8	4.4	3.7	3.8	3.7	4.1	
Relative root concentration ²		Percent 83	Percent 114	Percent 114	Percent 105	Percent 88	Percent 90	Percent 88		

¹ Percentage of total roots in top 4 feet.² Percentage of average concentration between 4 and 12 feet from trunk.

The lateral distribution of roots from the trunk is indicated by the average root concentration for all depths (based on averages for entire 12-inch depth increments) for each distance from the trunk. These averages show a slightly lower root concentration between 0 and 2 feet from the trunk and between 8 and 14 feet from the trunk than between 2 and 8 feet from the trunk. However, these differences are not great, indicating that roots penetrate soil zones in the middle of tree rows nearly as well as those zones nearer the trunk.

Relative root concentrations at each distance from the trunk are shown in the last line of table 3, with concentration expressed as percentage of the average of the concentrations between 4 and 12 feet. Thus the relative root concentrations in the 0- to 2-foot, 2 to 4-foot, 4- to 6-foot, 6- to 8-foot, 8- to 10-foot, 10- to 12-foot, and 12- to 14-foot zones are 83, 114, 114, 105, 88, 90, and 88, respectively. The relative rates of moisture extraction (table 1) at points 0, 2, 4, 6,

8, 10, 12, and 14 feet from the trunk were 120, 135, 110, 100, 100, 90, 100, and 100, respectively. Since root-distribution values are for soil zones 2 feet long, moisture extractions for zones, instead of for points, were estimated by averaging the relative moisture extraction at both ends of each zone. By this means relative moisture extractions for zones 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 14 feet from the trunk became 127.5, 122.5, 105, 100, 95, 95, and 100, respectively. These figures for relative root concentration and relative moisture extraction at each distance from the trunk show a zero correlation. This lack of correlation results principally from the lack of agreement between root concentration and moisture extraction in the 0- to 2-foot and 2- to 4-foot zones. Since moisture extraction at 0 feet from the trunk was based upon only one determination for each of the four depths in one sampling hole, the value 127.5 for relative moisture extraction for the 0- to 2-foot zone may be much less reliable than the other relative moisture-extraction values. With the 0- to 2-foot zone omitted, correlation between root concentration and moisture extraction becomes $+0.69 \pm 0.16$.

The root concentrations in table 3 show an unevenness in root distribution. Thus in the southwest trench in the upper 6 inches between 2 and 4 feet from the trunk, there were 4.1 g of roots per cubic foot, whereas in a similar soil zone in the northwest trench there were only 0.9 g of roots. Table 3 shows many such variations in distribution. Where the higher concentrations were not near large roots, it was not possible in the heavy clay soil to determine whether the root growth occurred at the end of vigorous lateral roots or whether it was stimulated as a result of the cutting of roots by cultivation implements, as the work of Kinman (7) suggests. However, regardless of the cause, the uneven root distribution observed around such a large tree in soil only $3\frac{1}{2}$ feet deep shows one very important point, namely, that penetration of the soil mass by the small roots (less than 2 mm in diameter) was not uniform.

Furthermore, it was observed that some small soil volumes contained no visible roots. It is not likely that roots too small to be observed had penetrated soil volumes in which no visible roots were found. Thus it would seem that in this heavy clay soil, root distribution is not only uneven but apparently entirely lacking in small volumes in the soil mass.

ROOT WEIGHTS FOR OTHER TREES

Additional data upon root distribution were provided by trenches dug on two sides of trees E-6 and L-12 at the Medford experiment station and on one side of trees E-4 and B-7 in the Klamath orchard. Each of these four trees had a much smaller spread of limbs than tree MM-33. The trenches were 18 inches wide and 36 inches deep. The roots were removed and weighed in a manner similar to that used for tree MM-33. The data for these four trees, together with comparable data for tree MM-33, are summarized in tables 4 and 5.

Table 4 gives the relative root concentration at each depth, thus expressing root concentration as percentage of the total of the root concentrations between the surface and a depth of 3 feet. Individual trees show rather large differences in relative root distribution, but the data for each tree are too limited to justify explanation on the

basis of tree size or soil management. The average for trees E-6, L-12, E-4, and B-7 and the average for tree MM-33 agree extremely well. The averages for all 10 trenches (5 trees) were determined and the probable errors of the averages were calculated on the assumption that the data for all trenches were comparable.

TABLE 4.—*Relative concentration of visible pear roots, under 2 mm in diameter, in the top 3 feet in heavy clay soil, from 3½ to 6 feet deep to bedrock*

[Relative concentration of roots is expressed as percentage of the sum of the concentrations in the top 3 feet]

Trench designation	Roots in soil zone at indicated distance below ground		
	0-1 feet	1-2 feet	2-3 feet
	Percent	Percent	Percent
Klamath B-7.....	48	34	18
Klamath E-4.....	23	40	37
E-6, N.....	35	36	29
E-6, S.....	35	36	29
L-12, N.....	53	30	17
L-12, S.....	45	37	18
Average.....	40	35	25
MM-33, SW.....	36	36	28
MM-33, SE.....	38	34	28
MM-33, NW.....	44	37	19
MM-33, NE.....	45	34	21
Average.....	41	35	24
Average of 10 trenches...	40.2	35.4	24.4
Probable error of average.....	±1.8	±0.6	±1.4

TABLE 5.—*Relative concentration of visible pear roots, under 2 mm in diameter, in the top 3 feet at different distances from the trunk, for heavy clay soil from 3½ to 6 feet deep*

[Relative concentration of roots is expressed as percentage of the average concentration between 4 and 12 feet from the trunk]

Tree designation	Roots in soil zone at indicated distance from trunk					
	0-2 feet	2-4 feet	4-6 feet	6-8 feet	8-10 feet	10-12 feet
	Percent	Percent	Percent	Percent	Percent	Percent
Klamath B-7.....	75	103	130	110	114	46
Klamath E-4.....		129	118	103	100	70
E-6, N.....	53	82	99	173	71	57
E-6, S.....		107	97	113	116	74
L-12, N.....	106	147	129	108	98	65
L-12, S.....		108	100	97	108	95
Average.....	78	113	112	117	101	69
MM-33, SW.....		103	99	108	92	101
MM-33, SE.....		140	111	117	93	79
MM-33, NW.....	76	127	123	108	76	93
MM-33, NE.....		91	125	90	91	94
Average.....	76	115	115	106	88	92
Average of 10 trenches...	¹ 78	114	113	113	96	78
Probable error of average.....	±7.3	±4.5	±2.9	±4.8	±3.2	±3.9

¹ Average of four trees.

To make the values for relative root concentration directly comparable with values for average relative soil-moisture extraction for each of 4 top feet (table 1), these relative concentrations for the top 3 feet were recalculated on the basis of the top 4 feet. The percentage concentration in the 3- to 4-foot depth was assumed to be 11, which was the observed concentration in the 3- to 4-foot zone for tree MM-33. Thus the relative concentration of roots in the 0- to 1-, 1- to 2-, 2- to 3-, and 3- to 4-foot depths would be 36, 31, 22, and 11, respectively. The average relative moisture extraction for similar zones (table 1) was 34, 28, 22, and 16, respectively. This average distribution of root concentration, based on 10 trenches around 5 trees, compared with the average relative moisture extraction observed at similar depths around comparable trees, shows the close correlation of $+0.98 \pm 0.01$.

Table 5 gives the relative root concentration, based on all depths, for each distance from the trunk. The results are expressed as percentages of the sum of the root concentrations between 4 and 12 feet. The averages for trees E-6, L-12, E-4, and B-7 agree fairly closely with the averages for the four trenches around tree MM-33. To further summarize the results, the average for all trenches is presented, together with the probable error of the average calculated on the assumption that all trenches were comparable. These averages show that the zones 2 to 4, 4 to 6, and 6 to 8 feet from the trunk had about equal root concentrations, the zone 0 to 2 feet from the trunk and the zones 8 to 10 and 10 to 12 feet from the trunk having lower concentrations.

From the average relative moisture extractions at points at different distances from the trunk (table 1), the average relative extractions for zones 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, and 10 to 12 feet from the trunk were calculated and found to be 87, 107.5, 107, 105, 101.5, and 91, respectively. A comparison of these relative moisture-extraction values with the relative root concentrations for similar zones (78, 114, 113, 113, 96, and 78, respectively) shows a correlation of $+0.96 \pm 0.02$.

DISCUSSION

The decrease in soil moisture between field capacity and permanent wilting percentage in heavy clay soil surrounding the roots of large pear trees represents either surface evaporation or extraction by pear roots. It was found from soil cores without active roots that surface evaporation affected moisture only in the top foot. When the soil moisture decreases in the top foot had been corrected for surface-evaporation loss, the soil moisture decreases were considered measures of the amount of moisture extracted by the roots.

Although it is probable that soil-moisture movement from the soil into the roots largely occurred through the root hairs and rootlets too small to be observed and weighed by the method used, it was assumed that the water-absorbing area of such root hairs and rootlets was approximately measured by the weight of the small visible roots picked out of the heavy clay soil.

It was found that the relative rates of moisture extraction by the roots at different soil depths and at different distances from the trunk were, in general, directly proportional to the relative concentrations of small visible pear roots in corresponding locations. In

the case of tree MM-33 no linear correlation was found between root concentration and rate of moisture extraction. However, the rate of extraction from the 0- to 2-foot zone is based in part on a single hole at the base of the tree. When the data for the 0- to 2-foot zone are omitted the coefficient of correlation is +0.69.

The correlation for tree MM-33 was +0.95 for all depths. However, when the averages of relative moisture extraction for the 4 individual trees and the 4 irrigation plots were compared with averages of relative root concentration for 10 trenches around an aggregate of 5 trees, the coefficient of correlation in relation to depth was +0.98 and the coefficient of correlation in relation to distance from the trunk was +0.96. Thus, when the averages were based on a sufficient number of determinations to reduce the influence of wide individual variations, the average relative moisture extraction was closely correlated with the average relative concentration of small visible roots. In other words, the rate of extraction of available moisture from the soil seems dependent upon the concentration of the small roots. Somewhat similar results were obtained by Conrad and Veihmeyer (5), who, working with a row of sorghum plants in relatively light soil, found a positive correlation between the extent of soil dryness and the amount of roots.

Beckett, Blaney, and Taylor (4), studying citrus and avocado in sandy loam 3 to 5 feet deep, report "apparent root development", as determined from the rate of water extraction, greatest in the first foot and materially decreasing with depth.

The positive correlation between the amount of soil moisture extracted from a specific soil mass and the pear root concentration therein also indicates that the weight of small visible roots per unit volume of soil provides a measure of root area active in water intake. Thus in regions where the transpiration needs of the trees are great, as in the Rogue River Valley, the water-supplying capacity of the roots should be judged by the distribution of zones of high root concentrations rather than by the presence of a few roots.

The fact that after moisture extraction by the roots different soil zones contained different amounts of moisture shows that capillary movement of moisture through the soil was not sufficient to equalize differences in rate of moisture removal by the roots. This is in agreement with the conclusions of McLaughlin (9), Veihmeyer (17), and others that capillary movement of moisture from soil relatively high in moisture to soil that is being dried out by root extraction is not sufficiently great to render all moisture in the soil mass equally available to the tree. The import for practical pear irrigation is obvious. In the determination of the time or amount of irrigation necessary for the trees, observations of soil moisture should be based on soil samples taken from zones of relatively great root concentration.

From the weights of the visible pear roots (less than 2 mm in diameter) per cubic foot of soil, it was found that the highest concentration of roots occurred between 4 and 12 inches below the surface. On the basis of four trenches around one large tree, it was found that of all the roots to a depth of 4 feet (bedrock $3\frac{1}{2}$ to 4 feet deep) 35 percent were in the top foot, 33 percent in the second foot, 21 percent in the third, and 11 percent in the fourth. Thus the top 2 feet contained 68 percent of the total roots in the upper 4 feet, and the upper 3 feet

contained 89 percent. These results agree with those obtained in extensive apple root examinations by Oskamp and Batjer (13), who found a greater concentration in the first 8 inches than in the second 8 inches and 60 percent or more of the roots in the top 16 inches; with those of Oskamp (12), who found cherry root concentration greatest in the top foot; and with those of Beckenbaugh and Gourley (3), who found that apple root concentration decreased from the surface down. Rogers and Vyvyan (16) found the same relation between depth and apple root concentration, except that the root concentration was greater between 17 and 44 cm below the surface than between 0 and 17 cm below the surface. More casual observations by Allen (1), Ballantyne (2), Lunt (8), Oskamp (11), and Partridge and Veatch (14) showed the greater portion of the root mass to be in the upper 1 or 2 feet. Such a relatively high proportion of the roots near the surface should be kept in mind when considering subsoiling, the depth to apply fertilizers, and the soil mass in which to maintain available moisture by irrigation.

As to the lateral distribution of small pear roots in 4 trenches around 1 large tree and in 6 trenches around a total of 4 other trees, the concentrations between 2 and 8 feet from the trunk were found to be slightly greater than those between 0 and 2 feet or between 8 and 14 feet from the trunk. However, the concentrations between 8 and 12 feet from the trunk, although in most cases significantly less than those between 2 and 8 feet, were much higher than those for apple roots in lighter soils. Furr and Magness (6), Morris (10), Rogers and Vyvyan (16), Rogers (15), and Beckenbaugh and Gourley (3) found that root concentration decreased appreciably with an increase in lateral distance from the trunk. Although Rogers and Vyvyan were studying rather small trees, the tree studied by Magness and Furr and the trees in orchard A examined by Beckenbaugh and Gourley were fully as large as the pear trees considered in this report. The large pear root concentrations at distances of 8 to 14 feet from the trunk in the heavy soil studied indicate that fertilizer and irrigation water for trees in this type of soil should, for availability to the maximum root area, be applied to the entire area between the tree rows.

SUMMARY

The amount of soil moisture extraction is positively correlated with the concentration of the small visible roots, which seems to be a measure of concentration of rootlets and root hairs too minute to be observed by the method reported.

Capillary movement of soil moisture is not sufficiently rapid to equalize differences in the amount of moisture extracted from different soil zones.

Root concentration is not uniform throughout the soil mass, and many small volumes of soil contained no visible roots.

Of the total moisture extracted from the upper 4 feet of soil, about 34 percent was extracted from the top foot, about 62 percent from the top 2 feet, and about 84 percent from the top 3 feet.

Of the total root concentration in the upper 4 feet of soil, about 35 percent was in the top foot, about 68 percent in the top 2 feet, and about 89 percent in the top 3 feet.

The most rapid moisture extraction by the roots occurred between 2 and 8 feet from the trunk, and 5 to 15 percent less rapid extraction occurred between 8 and 14 feet from the trunk.

The greatest root concentrations were found between 2 and 8 feet from the trunk, lower concentrations between 8 and 12 feet from the trunk.

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THE MEXICAN BEAN BEETLE IN MEXICO ¹

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INTRODUCTION

The Mexican bean beetle (*Epilachna corrupta* Muls.) is a destructive pest of beans in Mexico. Although this insect is apparently indigenous to the country and has a wide range of distribution, little is known

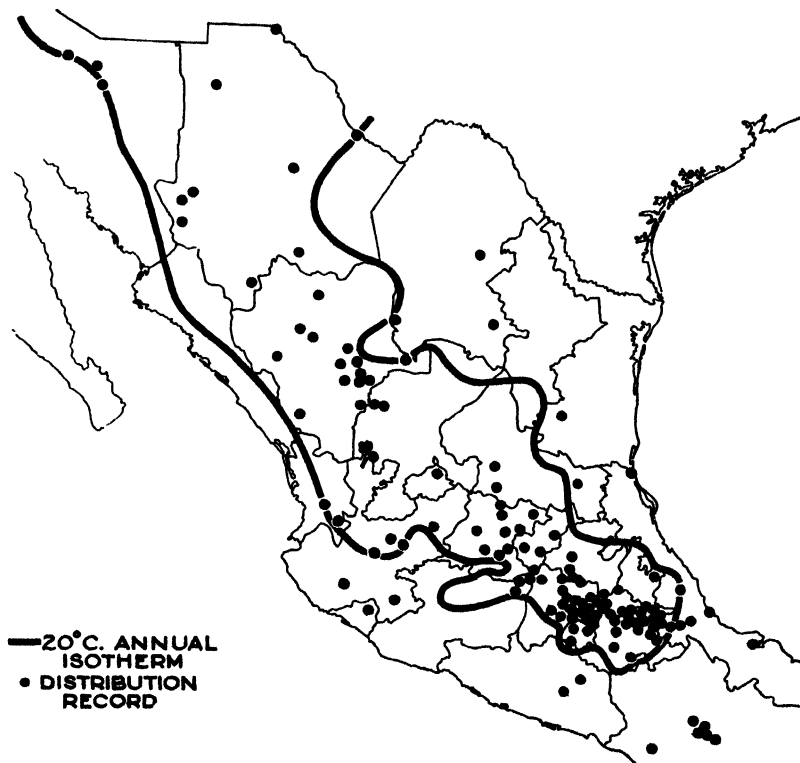


FIGURE 1.—Distribution of the Mexican bean beetle in Mexico.

concerning its biology. A study of the Mexican bean beetle was therefore undertaken at Mexico City in 1930.

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² Now with the Division of Fruit Insects.

³ Thanks are due Alfonso Dampf, of the Mexican Department of Agriculture, for assistance in procuring altitude records and to Leopoldo de la Barrera for making unpublished distribution records available.

DISTRIBUTION

Although many legumes are grown in Mexico, beans seem to be the preferred food plant of the bean beetle. In the States of Guanajuato, Zacatecas, Jalisco, Michoacán, Aguascalientes, Queretaro, Durango, Puebla, Veracruz, and San Luis Potosí, where commercial acreages of beans are grown, the bean beetle causes destruction to the crop periodically. Dampf (9) ⁴ says that, in all parts of the Republic where beans are cultivated, agriculturists know the bean beetle as the most terrible pest of this legume, occasionally causing complete loss of the crop. This is contrary to Sweetman's statement (11, p. 226) that "The insect is not a serious pest over much of Guatemala and Mexico * * *." Beans are grown from sea level to an elevation of approximately 10,000 feet, but the most extensive cultivation is found within these States, principally on the broad, central plateau having an elevation of 1,500 to 2,000 feet in the north and 7,000 to 8,000 feet or more in the Valley of Mexico or the vicinity of Mexico City (fig. 1). The 20° C. isotherm roughly depicts the limits of this plateau.

TABLE 1.—Localities in Mexico where the Mexican bean beetle has been recorded

Locality	Altitude	Reference ¹
	<i>Feet</i>	
Federal District:		
Contreras.....	Over 7,500.....	X.
Mexico City.....	7,349.....	(3), X.
San Jacinto.....	7,349.....	(7), X.
Tacuba.....	7,349.....	R.
Xochimilco.....	7,349.....	X.
Mexico (State of):		
Acozac.....	(3, 10).
Amecameca.....	8,189.....	X.
Apasco [Apaxco].....	(8, Bol. 2, p. 558).
Chalco.....	7,450.....	(10), X.
Chicoloapam.....	(10).
Hacienda del Prieto [Naucalpan].....	R.
Lechería.....	Over 7,000.....	R.
Naucalpan.....	R (Epilachna).
San Andrés Atenco [Tlalnepantla].....	Over 7,000.....	(8, Bol. 3, p. 501).
Temamatla.....	(10).
Temascalcingo.....	R.
Toluca.....	8,845.....	X.
Tenango.....	Over 8,000.....	(10, 1).
Texcoco.....	7,349.....	(10), X.
Zacualtipán, Estado General González.....	R.
Morelos:		
Cuautla.....	4,235.....	X.
Cuernavaca.....	5,059.....	X (1).
Puente de Ixtla.....	3,200.....	X.
Puebla:		
Acatzingo.....	R.
Actopan.....	(10).
Amozoc.....	(8, Bol. 2, p. 559).
Atlixco.....	6,171.....	(1), X.
Chalchicomula.....	8,333.....	(8, Bol. 2, p. 558).
Chilautzingo.....	7,516.....	(7).
Cholula.....	7,054.....	(1), X.
Cuetzalan del Progreso [Zacapoaxtla].....	R.
Huejotapá [Huejotapán].....	R.
Huixtola-Tecamachalco.....	6,742.....	R. (10, 3).
Malpás.....	(8, Bol. 2, p. 558).
Matamoros Juárez.....	4,350.....	(1).
Nopelucan, Hacienda de "La Florista".....	(8, Bol. 2, p. 332; 10).
Puebla.....	7,093.....	(1, 5), X.
Quecholac.....	R.
Rancho de San Cayetano, Tecamachalco.....	(5, 10).
San Cristóbal, Texmelucan.....	R.
San Hipólito.....	(8, Bol. 3, p. 245).

¹ Numbers refer to literature cited from which records were obtained, the volume and page number being given where confusion might arise; X indicates observations made by the authors; and R indicates records from the files of the Mexican Department of Agriculture.

⁴ Reference is made by number (italic) to Literature Cited, p. 1001.

TABLE 1.—Localities in Mexico where the Mexican bean beetle has been recorded—Continued

Locality	Altitude	Reference
	<i>Feet</i>	
Puebla—Continued.		
San Jerónimo Xayacatlán.....		R.
San Marcos.....		(10, 3).
San Martín Texmelucan.....		R.
Santa Ana Coapan [near Puebla].....		(8, Bol. 3, p. 245).
Tecali.....		(10, 3), R.
Tepatlaxco.....		R.
Tepeaca.....	7,405	(8, Bol. 2, p. 332).
Texmelucan.....		(8, Bol. 3, p. 501).
Tlaxpan.....		R.
Tlalancaleca.....		(8, Bol. 2, p. 848).
Veracruz:		
Córdoba.....	2,756	(1).
Jalapa.....	4,681	(1), X.
La Charca Atoyac.....	Nearly 1,500	R.
Orizaba.....	4,212	(1).
San Andrés Tuxtla.....	1,184	R.
Veracruz.....	6	(7, p. 248) ¹ .
Guerrero:		
Chilpancingo.....	4,461	(1).
Omiteme [Rancho de Chilpancingo?]		(1).
San Marcos.....		R.
Xucumanatlan.....		(1).
Oaxaca:		
Guelavía, Distrito Tlacolula.....	Over 5,000	R.
Juquila.....		(1).
Mutatlán, Distrito Tlacolula.....	Over 5,000	R.
Oaxaca City.....	5,068	(1), X.
Quiavini, Distrito Tlacolula.....	Over 5,000	R.
San Marcos Tlapasola, Distrito Tlacolula.....	Over 5,000	R.
Hidalgo:		
Bonintzá.....		(7).
Mixquiahuala de Juárez.....		R.
Rancho del Refugio Atotonilco de Tula.....		R.
Tapeji del Río.....		(7).
Tula.....	6,778	(8, Bol. 3, p. 501).
Queretaro:		
Cadereyta de Montes.....	6,814	R.
Queretaro.....	6,079	(3).
San Juan del Río.....	6,489	(7).
Michoacan:		
Aporo.....		R.
Maravatio.....	6,824	(10, 3).
San Andres Coru.....		R.
San Miguel el Alto.....		R.
Nayarit:		
Chapalilla.....		(8, Bol. 2, p. 559).
Tepec.....	3,067	(8, Bol. 3, p. 501).
Colima:		
Colima City.....	1,656	R (<i>Epilachna</i>).
Guanajuato:		
Allende [San Miguel Allende].....	6,135	R (<i>Epilachna</i>).
Celaya.....	5,931	R.
Dolores Hidalgo.....	6,519	R.
Guanajuato.....	6,725	(1; 8, Bol. 2, p. 559).
Hacienda de Carrancé [Cortazar].....		(3, 10).
Hacienda de Noria de Charcas, San José de Iturbide.....		(10).
Salamanca.....	5,646	(10).
San Diego de la Unión.....		(8, Bol. 2, p. 848).
San José de Iturbide.....		(10).
San Luis de la Paz.....		R.
Taranandacuau [Tarandacuao?]		R.
Jalisco:		
Guadalajara.....	4,987	X.
Hacienda de Las Pilas.....		(10).
Huejuquilla el Alto.....		R.
La Grulla Autlán.....		R.
Mezquital.....		R.
Orendain [near Refugio].....		(8, Bol. 2, p. 558).
San Martín Hidalgo.....		R. ¹
Villa de Guadalupe.....		(10, 3).
Aguascalientes:		
Rincón de Romos.....		(10).
San Luis Potosí:		
Estación Micos.....		(8, Bol. 2, p. 148).
Hacienda de Bledos [Bledos].....		(1).
Alvarez Mountains.....		(1).
San Luis Potosí.....	6,158	R (8, Bol. 3, p. 501).

¹Doubtful record. There is no certainty that *Epilachna corrupta* is the species to which reference is made.

TABLE 1.—Localities in Mexico where the Mexican bean beetle has been recorded—Continued

Locality	Altitude	Reference
	<i>Feet</i>	
Tamaulipas:		
Ciudad Ocampo.....	1,142.....	(10, 3).
Tampico.....	3.....	(11).
Durango:		
Barrazas.....	Nearly 6,696.....	(8, Bol. 3, p. 501).
Canacatlan [Canatlán].....	R.....	(7), R.
Durango.....	6,197.....	R.
Hacienda de El Salto [Pueblo Nuevo].....	Nearly 6,500.....	(10).
Nombre de Dios.....	(10).	(10).
Pánuco de Coronado.....	(10).	(10).
San Bartolo.....	(10).	(10).
San Bernardo el Oro.....	(10).	(10).
San Gabriel.....	(10).	(10).
San Juan de Guadalupe.....	Nearly 5,000.....	(10).
San Juan del Río.....	(10).	(10).
San Pedro Otáez.....	(10).	(8, Bol. 3, p. 501).
Santiago Papasquiaro.....	6,696.....	R.
Suchil.....	R.....	(8, Bol. 3, p. 501).
Tepehuanes.....	R.....	(8, Bol. 3, p. 501).
Villa General Vicente Guerrero.....	R.....	(8, Bol. 3, p. 501).
Coahuila:		
Monclova.....	1,758.....	(1).
Saltillo.....	5,246.....	(1).
Section around Torreón.....	3,641.....	(8, Bol. 2, p. 558).
Chihuahua:		
Batopilas [Batopilillas].....	5,105.....	R.
Casas Grandes.....	Nearly 4,777.....	R.
Chihuahua.....	1,564.....	(1, 8, Bol. 2, p. 558).
Chínipas.....	Over 5,000.....	R.
Ciudad Juárez.....	3,753.....	R.
Hacienda de San Simón Guadalupe y Calvo.....	R.....	R.
Hidalgo del Parral.....	5,449.....	R.
Ojinaga.....	2,759.....	R.
Yoquiva [Real Yoquiva].....	R.....	R.
Sonora:		
Atil.....	R.....	(7).
Cucurpe.....	R.....	(10).
Estación Esqueda.....	R.....	R.
Magdalena.....	2,595.....	(10).
Tlaxcala:		
Calpulápan.....	R.....	(1).
Contla.....	R.....	(7, p. 128) ² .
San Bartolo Tepujahualco [Tepeyahualco].....	R.....	R.
San Pablo del Monte.....	R.....	R.
Zacatecas:		
Canutillo.....	R.....	(8, Bol. 3, p. 501).
Sombrerete.....	R.....	(8, Bol. 3, p. 501).
Unknown localities:		
Hacienda del Rincón.....	R.....	(3).
Presidio.....	R.....	(1).

² Doubtful record. There is no certainty that *Epilachna corrupta* is the species to which reference is made.

Locality records from the Biologia Centrali-Americana (1), unpublished records and publications of the Mexican Department of Agriculture (7, 8, 9, 10), and field observations of the writers have shown the bean beetle to be present in 22 States and the Federal District (table 1). These records are not complete, but they show the wide distribution of the insect in the better known parts of Mexico. Unfortunately it has been impossible to procure altitude records for all the places in which the Mexican bean beetle has been found. However, it may be noted from the altitudes given that most of these places are from 5,000 to 7,000 or more feet above sea level. The extremes of elevation are 3 feet at Tampico, Tamaulipas, and 8,845 feet at Toluca, Mexico. Most of the records came from the States of Mexico, Puebla, Durango, Guanajuato, and Chihuahua, all of which are situated on the central plateau. Whether this is due to the fact that more beans are grown in these States and hence more

people report damage, or whether the damage actually is greater there than elsewhere, is not known.

Uvarov (12, p. 130) states: "This beetle is a serious pest in the parts of Mexico which have a hot and very damp climate * * *." However, with the exception of infestations reported from the vicinity of Tampico, Tamaulipas,⁵ Colima, Colima, and Veracruz, and possibly San Andres Tuxtla and La Charca Atoyac, Veracruz, the bean beetle does not appear to be present in the hot, damp coastal regions. Were it present or doing considerable damage, we should expect to find more records from the State of Veracruz, where commercial acreages of beans are grown.

SEASONAL ACTIVITY

At various times during the growing season of 1930 a few localities outside of the Federal District were visited in order that the situation as to bean beetle infestation might be ascertained. The results of these visits are summarized in table 2.

TABLE 2.—Mexican bean beetle infestation in various localities in Mexico on different dates in 1930

Date	Place	Elevation	Bean beetle situation
		Feet	
Apr. 28	Tampico, Tamaulipas ¹	3	No beans growing.
June 5	Veracruz, Veracruz ¹	6	Beans not infested.
6	Jalapa, Veracruz ¹	4,681	One adult beetle taken.
11	Puente de Ixtla, Morelos.....	3,200	Beetles found.
15	Monterrey, Nuevo Leon.....	1,771	No beans found.
17	San Luis Potosi, San Luis Potosi ¹	6,158	Do.
July 1	Cordoba, Veracruz ¹	2,756	Do.
4	Cuernavaca, Morelos ¹	5,059	All stages, severe damage.
9	Chilpancingo, Guerrero ¹	4,461	No beans found.
10	Acapulco, Guerrero.....	3	Do.
16	Cholulul, Puebla ¹	7,054	All stages; severe damage.
	Atlixco, Puebla ¹	6,171	Do.
Aug. 8	Guadalajara, Jalisco.....	4,987	Few larvae taken.
22	Oaxaca City, Oaxaca ¹	5,068	Young beetles and few larvae found.

¹ The bean beetle has been reported from this place (table 1).

In sections where there is a pronounced dry season, the bean beetle is present in the fields only during the wet summer months. There is considerable variation in annual precipitation within the area of known bean beetle distribution. In the vicinity of Jalapa and Cordoba it ranges from 60 to 85 inches and is distributed throughout the spring, summer, and fall. Around Torreon, in northern Mexico, the rainfall is only about 10 inches, and this occurs almost entirely during the summer months. In general, the period of summer rainfall is shorter and the precipitation less as the elevation and distance inland, northward, and westward from the Jalapa-Cordoba district increase.

A climograph (hythergraph) for Mexico City published by Graf (2) shows that the monthly temperature rises rapidly from January to May and declines during the 4 rainy months that follow. If temperature is the deciding factor in breaking the dormancy of the bean beetle, we should expect to find the beetle on beans grown under

⁵ ROBLEDO, F. G. OUTSTANDING ENTOMOLOGICAL FEATURES FOR MEXICO FROM JANUARY TO JUNE 1931. U. S. Dept. Agr., Bur. Ent. Insect Pest Survey Bull. 11:412-416. 1931. [Mimeographed.]

irrigation during April and May in Cuernavaca, since similar temperature changes occur there. The fact that the bean beetle does not appear in Cuernavaca and Mexico City until the summer rainy season indicates that moisture and increasing temperature furnish the stimulus required to bring the beetles from localities where they have passed the dormant period. The small summer crop of beans is grown in Cuernavaca without irrigation, and all the plants are defoliated by the beetle.

In 1929 some beans that had been planted in a protected spot at the Mexico City laboratory escaped the October frosts. Beetles and larvae migrated to these plants from other beans that had been killed by defoliation and frost. A sprinkler was used for several hours each day in order to maintain a high humidity. Beetles remained on these plants, and all stages of the insect were found there as late as February 3, when the observations were discontinued. This was 4 months after their normal occurrence in the field. Later work by the senior author in Columbus, Ohio, has shown that the bean beetle can easily be reared through the winter months under greenhouse conditions.

CLIMATE OF MEXICO CITY

According to Marcovitch and Stanley (5, p. 676):

During July and August, the months most favorable for bean growing, maximum temperatures of but 70° F. are reached in Mexico City (Hernandez, 1923). * * * May, the hottest month in the year, has a maximum temperature of but 75° F. These comparatively low temperatures and the generous rainfall of 16 inches for the summer months, are the climatic conditions the bean beetle has been exposed to for numberless generations and undoubtedly present the optimum requirements for breeding.

In another place (4) Marcovitch gives the mean summer temperature as 60° to 65° F. and the rainfall as 4 inches.

An atlas published by the Mexican Department of Agriculture (6) summarizes the temperature records^a taken at the Tacubaya (Federal District) station (about 4 miles from San Jacinto and at a slightly higher elevation) for the period 1921-25. The mean daily temperatures were as follows: March, 17.8° C.; April, 17.1° C.; May, 17.6° C.; June, 16.4° C.; July, 15.4° C.; August, 15.7° C. The maximum temperatures are not given, but the mean temperatures indicate that the maximum temperatures were higher than those given by Marcovitch and Stanley (5). The same atlas (6) shows that the total precipitation at the Tacubaya station, as averaged for 1921-25, was 411.4 mm for June, July, and August, and 582.2 mm for the 4 months from June to September, inclusive. The records for San Jacinto (6), within a quarter of a mile from the place in which the present studies were made, for the same periods were 356.5 and 492.3 mm, respectively.

Thus there are several conflicting records for the precipitation and temperature in the vicinity of Mexico City. This may be due to the fact that there are several meteorological stations in the Federal District, which might be designated as Mexico City, and the records of these stations vary considerably although they are less than 4 or 5 miles apart. It is, however, incorrect to base conclusions concerning the natural habitat of *Epilachna corrupta* on such information.

^a Obtained by taking the mean of the maximum and minimum daily temperatures and averaging them to get the monthly mean temperature.

In 1930 the writers placed a sheltered hygrothermograph 12 inches above ground in the middle of a bean field where life-history studies were being made. A summary of the temperature records taken on this instrument from July 1 to September 30, as given in table 3, indicates that the mean maximum temperature for the summer months was above 25° C. The weather that year was normal. The maximum daily temperature was recorded at about 2 p. m. and was maintained for but a short time. After the daily rain the temperature dropped rapidly until about 4 a. m.

TABLE 3.—Summary of daily temperature records at Mexico City (San Jacinto), July 1 to September 30, 1930

Record	July	August	September	For 3 months
	°C.	°C.	°C.	°C.
Maximum:				
High.....	30.0	30.0	34.5	-----
Low.....	23.0	20.0	25.0	-----
Mean.....	25.7	27.1	29.9	27.8
Minimum:				
High.....	14.0	13.0	13.0	-----
Low.....	8.0	7.0	2.5	-----
Mean.....	11.3	9.7	9.1	10.1
Daily mean.				
High.....	19.2	19.4	20.1	-----
Low.....	14.6	14.9	14.9	-----
Monthly mean.	16.7	17.2	17.7	17.2

¹ Summary of readings at 2-hour intervals.

The beginning of the rainy season of 1930, like that of 1929, preceded the emergence of the beetles from dormancy by approximately 2 weeks. During this season there was, as a rule, some precipitation every day, usually late in the afternoon or in the evening. The daily average relative humidity was close to 70 percent and showed little variation from early in June until the middle of September, when the rains became less frequent.

Other environmental factors, such as light and barometric pressure, may have more than a minor influence, directly or indirectly, on the bean beetle. At the altitude and latitude of Mexico City the sunlight is intense and contains more ultraviolet than at higher altitudes. At this altitude (7,349 feet) the mean barometric pressure is only 23.43 inches.

The writers consider that the field data obtained on the temperature and the number of generations a year do not indicate optimum climatic conditions for breeding the bean beetle. The low daily mean temperatures preclude the development of more than a single generation of *Epilachna corrupta* in the vicinity of Mexico City. Marcovitch and Stanley, after saying (5, p. 676) that climatic conditions in Mexico present "optimum requirements for breeding", also say (5, p. 769) that "At 25° C. the greatest percentage reached maturity, so that this temperature may be considered as the optimum." The work reported in this paper will show that 25° is more likely to be the optimum temperature than the temperatures that prevail in Mexico City.

EXTENT OF BEAN BEETLE INFESTATIONS IN VICINITY OF MEXICO CITY

The severest infestations in the vicinity of Mexico City in 1929 and 1930 were found in a large, dry lake bed between the villages of Mixquit and Chalco, 33 miles southeast of Mexico City. The soil here is alkaline and of a fine, silty texture. Mountains are not far distant. Each year this lake bed is planted to corn and interplanted with beans. There was considerable variation in the degree of infestation in this lake bed; in some places the plants were completely defoliated while in others there was little or no injury. It is estimated that less than half the plants showed any great injury. This variation did not seem to be due to any difference in the varieties of beans

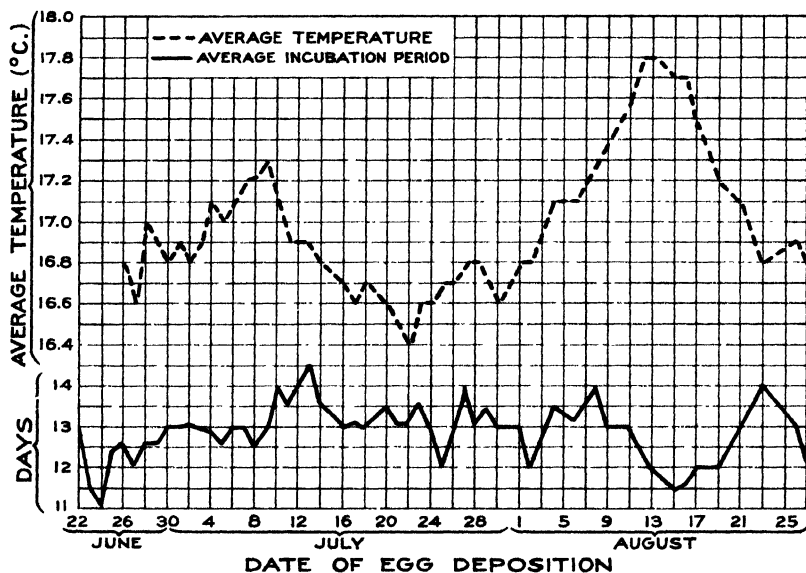


FIGURE 2.—Relation between the length of the incubation period of egg masses of the Mexican bean beetle and the temperature. The lower curve shows the average number of days required for the incubation of varying numbers of egg masses deposited each day during the season; the upper curve shows the average temperature during each of these incubation periods.

grown or to differences in soil or location. Other localities more remote from Mexico City showed more damage than was noted here.

LIFE-HISTORY STUDIES

Life-history studies were conducted in a field near the laboratory under conditions as nearly normal as possible. A wire-screen cylinder, 12 inches high and 8 inches in diameter, covered at one end, was inverted over a bean plant on which a pair of beetles had been placed. After an egg mass had been deposited on a leaf, the beetles were moved to another plant. Twelve pairs of beetles were kept under observation in this manner throughout the season. Each cage was observed daily, and records were continued until all the adults of the new generation had emerged.

OVIPOSITION

The first egg mass of the season was obtained the first week of June. The population of beetles in the field continued to increase until late in June, when the maximum number of adults that had passed the dormant period were found. The females continued to oviposit well into September, although by that time they were very much reduced in number. As the season advanced, the intervals between the successive depositions of egg masses became longer. A total of 148 egg masses was deposited, each mass containing from 5 to 60 eggs, the average being 49.73 ± 0.81 eggs.⁷

INCUBATION PERIOD

The length of the incubation period and its relation to temperature are shown in figure 2, in which are plotted the average time required for incubation of varying numbers of egg masses deposited on successive days and the average temperature during the incubation of egg masses deposited each day. For example, the average length of the incubation period of the 7 egg masses deposited on June 29 was 12.6 days, and the average daily mean temperature for this period was 16.9°C .

The incubation period is shown to range in length from 11.1 to 14.6 days. One egg mass, deposited on July 13, had an incubation period of 15 days, and 4 egg masses, deposited on June 23 and 24 and August 15 and 16, had incubation periods of 11 days, but such extremes were reduced by averaging them with records for other egg masses deposited on the same day. The average length of the incubation period for 148 egg masses was 12.94 ± 0.06 days. The average daily mean temperature for the season (June 26 to Sept. 7, inclusive) during which the incubation period was studied was 16.9°C . and the average relative humidity was 72.1 percent.

LARVAL PERIOD

In a similar way the length of the larval period and its relation to temperature are shown in figure 3. The average length of this period ranges from 36 days for eggs hatching on July 7 to 29.2 days for eggs hatching on August 14. The extremes, which are not shown when the average for the larvae in several cages is taken, are 37.2 days for an egg mass hatching July 4 and 28.0 days for an egg mass hatching August 14. The curve showing the length of the larval period has many irregularities that cannot be ascribed to variations in temperature but are probably due to the fact that the larvae may move about and may be shaded more or less by foliage on the plants. This means that the larvae are not always exposed to temperatures recorded on the thermograph. The average length of time spent in this stage of development, as determined from 127 cages containing 2,645 larvae, was 32.79 ± 0.14 days. The average mean daily temperature for the entire larval period, June 26 to September 30, was 17.2°C .; the average relative humidity was 69.7 percent.

⁷ Throughout this paper the standard error of the mean is used and not the probable error.

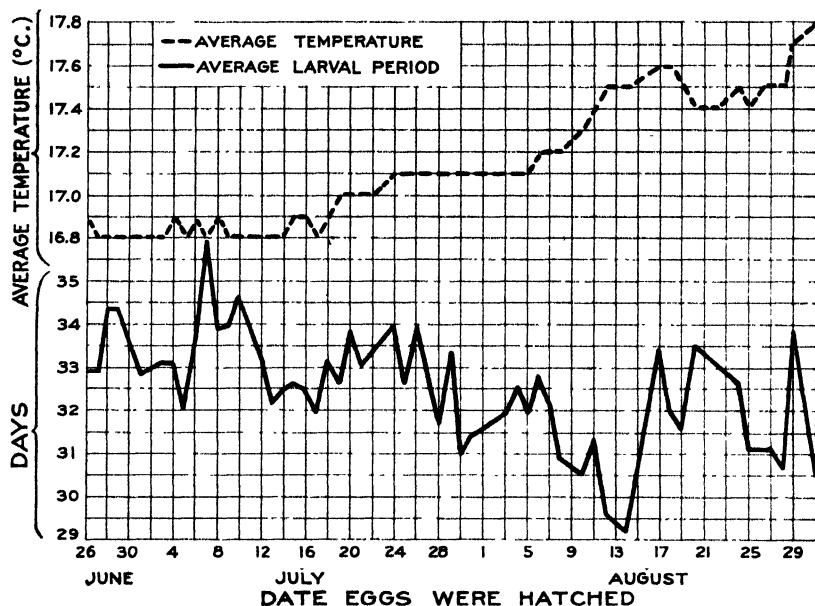


FIGURE 3.—Relation between the length of the larval period of the Mexican bean beetle and the temperature. The lower curve shows the average number of days required for the larval period of individuals from egg masses hatching each day during the season, the upper curve shows the average temperature during each of these periods.

The data concerning the length of time spent in the several instars, the prepupal period, and the total larval period are shown in table 4.

TABLE 4.—Length of instars and prepupal and larval periods of *Epilachna corrupta*, San Jacinto, 1930

Instar and period	Cages	Individuals	Length of period, based on the cage as a unit		
			Maximum	Minimum	Average
	Number	Number	Days	Days	Days
Entire larval period.....	127	2,645	37.2	28	32.79±0.14
First instar.....	96	2,667	10	6	7.75±.09
Second instar.....	90	2,463	9	5	6.12±.08
Third instar.....	81	2,144	9	5	6.71±.12
Fourth instar less prepupal period.....	70	1,421	11	6.9	8.45±.11
Fourth instar including prepupal period.....	78	1,605	15.7	9	12.24±.12
Prepupal period.....	122	2,556	5.3	2.2	3.9±.05

PUPAL PERIOD

The length of the pupal period and its relation to temperature are shown in figure 4. The length of this period ranged from 9 to 12.6 days, but there were extremes of 13 days (individuals entering this period on Aug. 27) and 9 days (individuals entering this period on Sept. 22) when separate cages are taken into account. The average for 119 cages containing 2,090 pupae was 11.33 ± 0.08 days. The average daily mean temperature for the time pupae were studied (July 26 to Oct. 4, inclusive) was 17.4°C .; the average relative humidity, 67.3 percent. Since the pupal period is shorter and the pupae

remain fixed on the under surface of the leaves, the trend in temperature and its relation to the length of the pupal period are shown more clearly than for either the incubation period or the larval period. There are, however, several departures from the general trend that cannot be explained on the basis of temperature.

ENTIRE DEVELOPMENTAL PERIOD

One generation of *Epilachna corrupta* developed at Mexico City (San Jacinto) in an average of 56.74 ± 0.21 days. The extremes were 61.8 and 50 days. These records represent 1,687 beetles contained in 94 cages. The average temperature for the 102-day period from June 26 to October 5, inclusive, was 17.2°C. , and the average relative humidity was 66.6 percent. A total of 2,097 adults, including

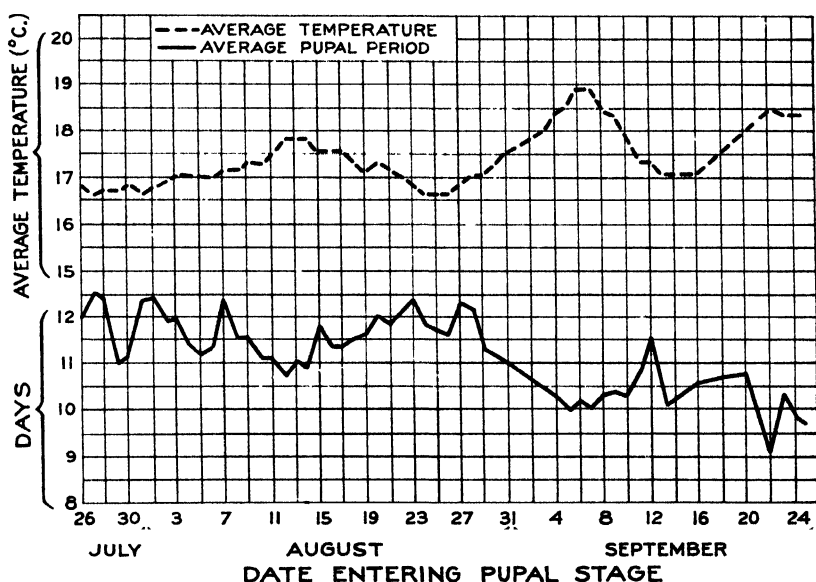


FIGURE 4—Relationship between the length of the pupal period of the Mexican bean beetle and the temperature. The lower curve shows the average number of days required for the pupal period of larvae entering this stage each day during the season, the upper curve shows the average temperature for this period.

some whose life history was not recorded, emerged during this period. Of these, 975, or 46.5 percent, were males. The first adults of the season appeared July 26, 1930. Marcovitch and Stanley (5) determined the length of the developmental period of the bean beetle in the laboratory at four constant temperatures. If a curve is drawn to represent their results at these temperatures (fig. 5), the writers' figure for the total period of development from egg to adult (56 days) will pass through this curve.

BEHAVIOR LATE IN THE SEASON

Seven female and six male beetles that emerged August 8 were placed in a cage for observation. The beetles fed ravenously, but there was no oviposition until August 26, when a mass of 5 eggs was recorded. Four scattered eggs were laid September 5, and a mass of

31 was deposited September 12. The beetles consumed less and less foliage, and in the second week of September they ceased feeding altogether. Thereafter they remained in a state of torpor in protected places on the plant. The beetles were completely bronzed during this period.

OVERWINTERING

Beetles can be found in the fields until the frosts of early October destroy the plants. It is a matter of conjecture where the beetles pass the dry season from October until June. E. G. Smyth⁸ reports the natives as saying that swarms of adults were seen to rise from the fields when there was no longer food and that they were carried off by the wind. Alfonso Dampf found an adult bean beetle in wheat stubble on the grounds of the Mexican Department of Agriculture in the winter of 1928.

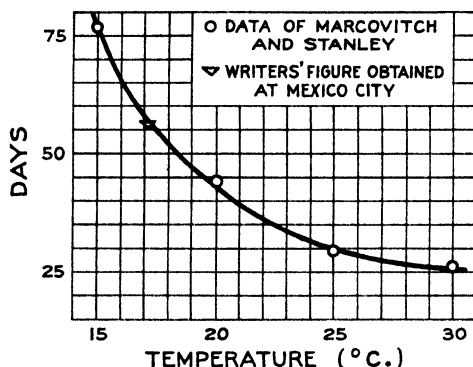


FIGURE 5.—Relationship between the length of the total developmental period of the Mexican bean beetle at constant temperature as determined by Marcovitch and Stanley (5) in Knoxville, Tenn., and also as determined by the present writers under field conditions in San Jacinto, Mexico

SUMMARY AND CONCLUSIONS

The Mexican bean beetle (*Epilachna corrupta* Muls.), a widely distributed and destructive pest of beans in Mexico, has been recorded from elevations ranging from 3 to 8,845 feet, chiefly within the area delimited by the 20° C. isotherm, which includes most of the central plateau of Mexico. Precipitation and extremes of temperature within this area vary considerably. The effects of these factors on the development of the bean beetle have been determined only in Mexico City.

Severe damage caused by this insect was observed at Atlixco, Puebla (6,171 feet), and at Cuernavaca, Morelos (5,059 feet). In the vicinity of Mexico City the heaviest infestation was found near the villages of Mixquit and Chalco, but less than half of the plants showed any serious injury.

Life-history studies made near Mexico City under field conditions show the length of time spent in each developmental stage throughout the season and its relation to the temperature. One generation of bean beetles matured in an average of 56.74 ± 0.21 days at a mean

⁸ Correspondence with N. F. Howard, 1923.

temperature of 17.2° C. and an average relative humidity of 66.6 percent.

Apparently the destruction of the bean plants by frost has much to do with the disappearance of the adult insects from the fields in the fall. In one instance beetles continued to feed and oviposit 4 months beyond the time of their natural occurrence in the field. This was probably due to the favorable temperature and humidity maintained in a protected spot where beans were grown. It is not known where and how the adults pass the dry season in Mexico.

Optimum conditions for the bean beetle are not found in the Valley of Mexico, if we consider temperature, number of generations, and injury done to beans.

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